FORMULATION DEVELOPMENT AND NON-INVASIVE *IN-VIVO* EVALUATION OF COSMETIC EMULSIONS CONTAINING VARIOUS BOTANICAL EXTRACTS

A Thesis submitted in partial fulfillment of the requirements for the Degree of

Doctor of Philosophy
(Pharmaceutics)

By

Muhammad Khurram Waqas
B. Pharmacy, M. Phil.

Department of Pharmacy

Faculty of Pharmacy & Alternative Medicine

The Islamia University of Bahawalpur Pakistan

2012-2015

In the name of Almighty ALLAH the most beneficent, the most merciful

Dedicated to

My Parents

My mother: *Mumtaz Begum*

My father: *Ch. Saifullah (Late)*

It's all because of their Prayers

Table of Contents

LIST OF TABLES

LIST OF FIGURES

List of Abbreviations

Abbreviations	Meanings
UV	Ultraviolet
W/O	Water in oil
O/W	Oil in water
DPPH	2,2-diphenyl-1-picrylhydrazyl
SELS	Surface Evaluation of Living Skin
ANOVA	Analysis of variance
SEr	Skin roughness Evaluation
SEsc	Skin scaliness Evaluation
SEsm	Skin smoothness Evaluation
Sew	Skin wrinkles Evaluation
HLB	Hydrophilic Lipophilic Balance
O/W/O	Oil in water in Oil
W/O/W	Water in oil in Water
STI	Soybean trypsin Inhibitor
BBI	Bowman-Birk Inhibitor
GSPE	Grape seed proanthrocyanidine extract
OPC	Oligomeric Proanthrocyanidines
CIDS	Cholistan Institute of Desert Studies
SB	Soybean Base
SF	Soybean Formulation
GB	Grape seed Base
GF	Grape seed Formulation
TB	Tamarind Base
TF	Tamarind Formulation
SEM	Standard Error of Mean
SD	Standard Deviation
RH	Relative humidity

List of research articles published/accepted from this Dissertation work in HEC recognized /Impact factor journal

1. Screening of Various botanical extracts for antioxidant activity using DPPH free radical method. African Journal of Traditional Complementary and Alternative medicines. 10(6); 452-455: 2013. Impact factor: 0.56

2. Stability study of a cosmetic emulsion loaded with *Tamarindus indica* seeds extract. Latin American Journal of Pharmacy. 33 (5); 731-738: 2014. Impact factor: 0.319

3. *In vivo* evaluation of a cosmetic emulsion containing soybean extract for anti-aging Tropical Journal of Pharmaceutical Research. 13 (9);1401-1406: 2014. Impact factor: 0.495

4. Physical Stability, rheological analysis and antioxidant study of cetyl dimethicone copolyol based cosmetic water-in-oil emulsions. Latin American Journal of Pharmacy. 33 (10): (2014) Impact factor: 0.319

5. Dermatological and cosmeceutical benefits of *Glycine max* (soybean) and it's active components. Accepted for Publication in Acta poloniae pharmaceutica. Impact factor: 0.693

6. Devolpment of cetyl dimethicone based water-in-oil emulsions containing botanicals: Physical characteristics and stability. Accepted for Publication in Pakistan Journal of Pharmaceutical Sciences. Impact factor: 0.95

7. Cosmeceutic effects of grape seeds: a review. Accepted for Publication in Tropical Journal of Pharmaceutical Research. Impact factor: 0.495

Certificate

It is hereby certified that work presented by Muhammad Khurram Waqas S/O Ch. Saifullah in the dissertation entitled *"Formulation development and non-invasive in-vivo evaluation of cosmetic emulsions containing various botanical extracts."* has been successfully carried out under my supervision in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Pharmaceutics) under my supervision in the Department of Pharmacy, Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur.

Prof. Dr. Naveed Akhtar
Supervisor,
Faculty of Pharmacy and Alternative Medicine,
The Islamia University of Bahawalpur

Acknowledgements

Imperatively, I should bow to **ALLAH ALMIGHTY** who is the creator of the all creations. All respects for my Holy Prophet **MUHAMMAD (SAAW)**, who guided me to recognize my creator.

It is an immense pleasure to give my best gratitude to my research guide and enthusiastic supervisor, Prof. Dr. Naveed Akhtar (Chairman, Department of Pharmacy, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Pakistan) for his professional guidance, kind behavior, valuable time and scientific support. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined of having a better advisor and mentor for my Ph.D. study. I also obliged to Prof. Dr. Mahmood Ahmad (The Dean, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Pakistan) for precious advises during the study to facilitate my research work and helped me in providing all the facilities for the completion of this project. I appreciate the kind help of Dr. Muhammad Nadeem Ashraf, Dr. Haji M. Shoaib Khan, Dr. Akhtar Rasul, Dr. Barkat Ali Khan and Dr. Ghulam Murtaza for their guidance from time to time. I shall not forget the support of my lab fellows, Rehan Mustafa, Masood aarbi, Irfan Aslam, Sabeeh Mohsin, Hira Khan, Muhammad Sohail, Shakeel Ijaz and Atif Meo. I shall remember their teamwork and sincerity forever.

Above all, I extend my everlasting gratitude and love to my father (who sadly passed away last year) and mother who prayed for me and to my sister, wife and children, who supported and encouraged me all the way with their incredible patience. Only their continuous care, unconditional love, and incomparable sacrifice made all this possible, and easy to achieve.

My deep gratitude is extended to all those who helped me in any form throughout this journey.

MUHAMMAD KHURRAM WAQAS

Declaration of Originality

I, Muhammad Khurram Waqas, Ph.D. Scholar of the Department of Pharmacy, The Islamia University of Bahawalpur, hereby declare that this research work entitled *"Formulation development and non-invasive in-vivo evaluation of cosmetic emulsions containing various botanical extracts"* is done by me. I also certify that nothing has been incorporated in this dissertation without acknowledgment and that to the best of my knowledge and belief it does not contain any material previously published or any material previously submitted for a degree in any University; where due reference is not made in the text.

Muhammad Khurram Waqas

Abstract

The apparent symptoms of skin aging are the result of triggering of free radicals by ultraviolet (UV) light from sun. The conventional therapy composed extremely of herbal formulations to have juvenile, elegant and youthful skin. For the maintainace of proper functions of human skin antioxidants are very vital. For topical application on visual human skin like cheeks, cosmetic W/O emulsions loaded with botanical extracts from various plants were developed by using fewer ingredients and non-invasive *in vivo* evaluation is on various skin related parameters was performed. The preparation of concentrated botanical extracts from various plants namely tamarind (*Tamarindus indica*), grape seeds (*Vitis vinifera*) and soybean (*Glycine max*) was done by utilizing ethyl alcohol and n-hexane as solvents. These extracts were detected for the antioxidant activity by using DPPH method. Cosmetic water-in-oil emulsion was developed by heating oily and aqueous at 75ºC ±1 ºC and then dispersion of aqueous phase in oily phase was carried out with persistent stirring. The incorporation of botanical extract in aqueous phase before addition in oily phase was done. The preparations of different bases lacking extracts were prepared to be used as control. The emulsions were monitored for stability for the duration of twelve weeks. The evaluation of viscosity, phase separation, liquefaction and color was done by keeping cosmetic emulsions at different conditions of storage at 8 ± 0.5°C, 25± 0.5°C, 40± 0.5°C, and at 40°C ± 0.5°C with 70% RH (relative humidity). The predicted stability of cosmetic emulsions was attained from three months *in-vitro* study duration. After obtaining written informed consent, selected cosmetic emulsion formulations and their respective bases were handed over to thirty three (11 for each active formulation) healthy male human volunteers for application on respective cheeks for a duration of twelve weeks. Surface evaluation of living skin (SELS) was evaluated by availing Visioscan® VC 98 initially and after application of cosmetic emulsions. Furthermore by the help of Corneometer, Mexameter, Sebumeter and Elastometer, the evaluation of various skin parameters was carried out. By applying paired sample t-tests and two-way analysis of variance (ANOVA) results were assessed statistically. The significant effects ($p \leq 0.05$) produced by the all active cosmetic emulsions formulations on SELS (Surface evaluation of living skin) parameter for example skin roughness (*SEr*), skin scaliness (*SEsc*), skin smoothness (*SEsm*) and skin wrinkles (*SEw*).The results was strengthened by three dimensional images . Skin elasticity and skin moisture contents were increased significantly supporting the results of parameters of surface evaluation of living skin. A statistically significant difference ($p \leq 0.05$) was assessed for active cosmetic formulations concerning melanin, erythema and skin sebum contents as compared with base formulations. The conclusion of this work is that the stable active cosmetic emulsions formulations containing various botanical extracts exerted significant effects on various skin parameters such as skin whitening effects, anti-acne effects, antiaging effects, and alleviation of skin dryness and improvement of skin complexion.

Key Words: *Tamarindus indica* ,*Vitis vinifera* , *Glycin max*, Erythema, Melanin, Skin sebum, Skin moisture contents, Surface evaluation of living skin (SELS).

Abstract (Urdu)

سورج سے نکلنے والی بالائی بنفشی (UV)روشنی کے جلدپر پڑ نے سے آزاد ذرات (Free Radicals) بنتے ہیں جو جلدی بڑھا پے کا باعث ہوتے ہیں۔ روائتی طریقہ علاج میں جلد کو بے مثال ، خوبصورت اور نوجوان رکھنے کیلئے کئی نباتاتی مرکبات موجود ہیں۔ انسانی جلد کی مناسب بحالی کے لیے اینٹی آکسیڈنٹ (Antioxidant)بہت ضروری ہیں ۔ جو مختلف پودوں میں کثیر تعداد میں موجود ہوتے ہیں ۔ موجودہ تحقیق میں جلد پر استعمال کے لیے پودوں سے حاصل شدہ نباتاتی عرق سے بھرپور کریمیں تیار کی گئیں جن کو رخساروں پر لگا کر مختلف معیاروں پر پرکھا گیا ۔ سویا بین (*Glysine max*) ، انگوروں کے بیج (*Vitis vinifera*) اور املی (*Tamaridus indica*)کا عرق تین مختلف کریموں میں ڈالا گیا۔ نباتاتی عرق کی تیاری کے لیے ایتھا ئل الکوحل(Ethanol) اور این ہیگذین (N-Hexan) بطور محلول استعمال ہوئے۔ ان عرقوں میں جلد کو جوان رکھنے کے لیے بھرپور اینٹی آکسیڈنٹ (Antioxidant)موجود تھے جن کی تصدیق DPPHکے تجزیے سے ہوئی۔ ان کریموں کی تیاری کے مراحل میں پانی اور تیل کے اجزا کو 75ڈگری سینٹی گریڈ پر گرم کرنے کے بعد برقی مد ھانی(Stirrer) میں مسلسل ہلا یا گیا ۔ پانی والے اجزا کو تیل والے اجزا میں ملانے سے پہلے پانی میں نباتاتی عرق ملا دیا گیا تھا۔ مختلف کریمیں جن کو بیسز (Bases) کہا جاتا ہےنباتاتی عرق کے بغیر تیار کی گئیں ۔ ان کریموں کو بنانے کا مقصد یہ فرق معلوم کرنا تھا کہ آیا کون سی کریم جلد پر زیادہ اثرات مرتب کرتی ہے۔ کریموں کی پائیداری کو جانچنے کے لیے ان کو مختلف درجہ حرارت اور حالات میں تین مہینے تک زیر نگرانی رکھا گیا۔ کریموں کو رکھنے کے لیے مختلف انکیوبیٹر ز(Incubators)استعمال کیے گئے جن کو مختلف درجہ حرارت ($8C^o,25C^o,40C^o,40C^o$ + 75% RH) پر سیٹ کیا گیا تھا۔ تین مہینوں کے دوران مختلف اوقات میں ان کریموں کا گاڑھاپن (Viscosity)، اجزا کی علیحدگی (Phase Separation)،پتلاہٹ (Liquefaction) اور رنگ (Color) کی جانچ کی گئی ۔ کریموں کی پائیداری جانچنے کے بعد انسانی جلد پر ان کے اثرات کی تحقیق کے لیے تینتیس صحت مند مرد رضا کاروں کا انتخاب ان سے تحریر ی اجازت نامہ حاصل کرنے کے بعد کیا گیا ۔ ہر کریم کے لیے گیار ہ رضاکار منتخب کیے گئے جن کو نباتاتی عرق والی اور نباتاتی عرق کے بغیر والی کریمیں فراہم کی گئیں ۔ ان میں سےہر ایک رضا کار کو نباتاتی عرق والی اور نباتاتی عرق کے بغیر والی کریم رخسار کی مخصوص جگہ پر روزانہ لگانے کی ہدایت کی گئی ۔ مختلف جلد ی آلات کی مدد سے ان کریموں کا جلد پر اثر ات کا مشاہدہ کیا گیا ۔ جلد کے کھردرے پن ، جھریوں، داغ دھبوں اور ملائمت کو جدید آلے (Visioscan VC98®)کی مدد سے کریمیں لگانے سے پہلے اور بعد میں پرکھا گیا۔ مختلف جلدی آلات مثلاًمیگذا میٹر (Mexameter®)، کا رنیومیٹر (Corneometer®)، سیبو میٹر (Sebumeter®) اور الاسٹو میٹر (Elastometer®) کی مدد سے جلد کے مختلف معیاروں کو مزید جانچا گیا۔ پیرڈ سامپل ٹی ۔ ٹیسٹ (Paired Sample t-Test) اور ٹو ۔وے انووا (Two-Way ANOVA) کے اطلاق سے نتائج کے اعداد و شمار معلوم کیے گیے ۔ نباتاتی عرق سے بھرپور کریموں نے چہرے کی جھریوں ، داغ دھبوں اور کھردرے پن کو دور کرنے اور جلدی ملائمت ، نمی اور جلدی لچک بڑھانے میں واضح نتائج ظاہر کیے ۔مزید برآں تین جہتی تصویروں (Three Dimensional Images)نے نتائج کو مزید مئوثر بنادیا۔ میلانن (Melanin)، سیبم (Sebum) اور اردما (Erythema) کے اعدادو شمار میں واضح کمی دیکھنے میں آئی ۔ الغرض اس تحقیق کا نچوڑ

ہمیں یہ واضح کرتا ہے کہ نباتاتی عرق سے بھرپور کریمیں جلد کے مختلف معیاروں مثلاً جلدکا گوراپن ، داغ دھبوں ، جھریوں ، دانوں جلد کے کھردرے پن اور رنگ روپ میں نکھار پر واضح اثرات مرتب کرتی ہیں۔

CHAPTER 1

INTRODUCTION AND AIMS

1. Introduction and Aims

1.1. Introduction

The use of botanical extracts in cosmetic formulations is increasing day by day. Botanical extracts that support the health, texture and integrity of the skin, hair, and nails are widely used in cosmetic formulations. Natural remedies have been used for centuries to treat skin conditions and a variety of dermatological disorders including inflammation, phototoxicity, psoriasis and atopic dermatitis (Zhu and Gao, 2008). Over the past decade, there has been fervent interest in products found in nature because of their perceived safety. Skin care products are often developed from plants. Many believe that if a product can be safely ingested, it will also be safe for topical application. Botanical extract for topical applications are considered safe by US food and drug administration, thus allowing the products to be marketed without obtaining status or being restricted by monographed ingredients (Antignac et al., 2011).

Today cosmetic formulators have access to plant material worldwide for incorporation into cosmeceuticals (Thornfeldt, 2005). In general, plant-derived, botanical cosmetic products tend to be antioxidant in action since these organisms must thrive in constant direct ultraviolet (UV) light, the Earth's most prolific manufacturer of free radicals. The botanical originated antioxidants rather than artificially produced ones are supposed to be favorable for preservation of proficient health conditions (Reuter et al., 2010). There is profound indication that antioxidant can assist to foil and even revocation of some of the consecutions of skin aging. Antioxidant potential is an outstanding case of practical advantages that botanical extract can provide. Numerous botanical extracts and components isolated from botanicals are frequently availed in cosmeceuticals for their strong antioxidant potential and are auspicious candidates for lessening the effects of aging progression on skin by restraining oxidation (Afaq et al., 2002). The positive perceptions about the use of natural antioxidants by consumers make them chiefly important cosmetic ingredients (Ditre et al., 2008). New botanical skin care treatments are emerging, presenting dermatologists and their patients the challenge of understanding the science behind these cosmeceuticals (Graf, 2010).

Botanical extracts of various plants were used in this study. It is of utmost importance for *in-vivo* studies related to skin and cosmetic research to specifically quantify various skin physiological parameters using noninvasive methods. For this purpose, several bioengineering techniques have been developed to detect and measure early skin damage, which is relatively difficult to express clinically.

Emulsions are thermodynamically unstable systems which split into two distinguishable phases. The instability is manifested by a number of processes such as flocculation, sedimentation or creaming, phase inversion or coalescence that would destabilize them. In order to disperse two immiscible liquids a third component is required, namely the emulsifier; the choice of emulsifier is crucial not only for the formation of the emulsion but also for its long-term stability (Nielloud, 2000). Oil-in-water or water-in-oil emulsions are the examples of colloidal systems that are frequently used now a day in various fields as pharmaceuticals, cosmetics, paints, food and petrochemicals etc.All these emulsions evolve gradually with time (Lieberman et al., 1998). A wide variety of cosmetic emulsions are used as bases for skincare products for healthy and diseased skin. These products can range in consistency from a cream to a lotion or body milk and even a fluid for normal, oily or dry skin. Since today's trends cosmetic products needs to be in accordance with the new market demands. This means that development times must be drastically reduced while maintaining the same high product quality. So keeping in mind all this, while developing cosmetic products quickly and effectively, manufacturer needs reliable methods to obtain product stability without the need of long time testing (Eccleston, 1997).

Water-in-oil emulsions consists of the water phase ,which is internal /dispersed phase, mixed with oil, which is continuous phase .This emulsion type is often more difficult to prepare and stabilize since it is most often based on totally non- ionic emulsifiers. However recent advances in silicon chemistry and polymer chemistry have allowed the preparation of excellent water-in-oil (W/O) emulsions. A real benefit of these vehicle emulsions is that they are readily spread on to the lipophilic skin and provide a film which is resistant to water wash off. This is how water resistant moisturizing cosmeceuticals are created. Since the emulsifiers used for these emulsions are lipophilic, meaning oil loving, they do not upset the lipid bilayer and thus will not damage the skin barrier. The W/O systems are formed, using nonionic emulsifier, only in a temperature range above the HLB temperature, where the oil is expected to be a continuous phase (Tal-Figiel, 2007). A wide variety of emulsifiers are used in pharmacy and cosmetics to prepare cosmetic emulsions. Nevertheless, these emulsifiers are often responsible for allergies and irritations. Therefore it is very important to develop formulations of cosmetic emulsions with emulsifier that do not cause allergies and irritations (Fomuso et al., 2002). A non-ionic emulsifier that is Polysiloxane polyalkyl polyether copolymer commonly known as ABIL EM 90® has been used as an emulsifying agent, which finely distribute the water droplets into the continuous oil phase. ABIL EM 90® is clear and viscous oil soluble liquid having the HLB value equal to 5. It is widely used as emollient and anti-foaming agent in the cosmetic emulsions. It has high compatibility with active ingredients and form very stable formulations (Tamburic et al., 1996).

In this research botanical extracts of three plants namely tamarind (*Tamarindus indica*), grape seeds (*Vitis vinifera*) and soybean (*Glycine max*) containing various antioxidants were utilized in the formulation of cosmetic emulsions using non-ionic surfactant Abil EM90 to evaluate different skin parameters. Stability analyses were conducted by allowing the cosmetic emulsions to varying humidity and temperature conditions. Various parameters of *in -vitro* studies like phase separation, liquefaction, electrical conductivity, color, viscosity were also monitored to evaluate the stability of cosmetic emulsions. The appropriate bases containing no botanical extract were also formulated and were applied to male human volunteers for a period of three months along with emulsions containing botanical extracts. Various biophysical techniques were employed for the non-invasive *in-vivo* evaluation of various skin physiological parameters like skin elasticity, skin wrinkles, skin moisture contents, skin sebum contents, skin melanin and skin erythema. Data obtained were analyzed statistically.

1.2 Aims of the study

1. To formulate the cosmetic emulsions containing various botanical extracts
2. To assess the stability studies of these cosmetic emulsions.
3. To evaluate the efficacy of the selected botanical extracts for their claimed utilities on various skin physiological parameters .

CHAPTER 2

REVIEW OF LITERATURE

2. Review of literature

2.1. Emulsions

Emulsions are a class of disperse systems consisting of two immiscible liquids .The liquid droplets (the disperse phase) is dispersed in a liquid medium (the continuous phase). The terms emulsion and creams refer to disperse systems in which one insoluble phase is dispersed as droplets within a second liquid phase. Emulsions are inherently unstable systems; only the presence of an emulsifying agent compound enables them to persist in the dispersed state (Remington et al., 2006). The droplets of the dispersed phase are polydisperse spherical particles formed by subjecting the emulsion components to a milling or comminution process. Given the free energy associated with the immiscible liquid interface, the concomitant substantial increase in the interfacial area results in a thermodynamically unstable system which tends to revert back to the original two phase system with its minimum interfacial area (O'May et al., 2004). Amphiphilic molecules added to the system migrate preferentially to the immiscible liquid interface. Their interfacial adsorption is accompanied by a lowering of interfacial tension and a rise in interfacial viscosity. The net effect is an increase in the effective stability of the emulsion (Rousseau, 2000).

The particle diameter of the disperse phase generally extend from about 0.1 to 10µm, although particle diameters are as small as 0.01µm and as large as 100µm are not uncommon in some preparations . A more common average droplet size is 0.5-5.0µm (Vyas and Khar, 2004).

Several processes relating to the breakdown of emulsions may occur on storage, depending on:

- The particle size distribution and the density difference between the droplets and the medium.
- The magnitude of the attractive versus repulsive forces, which determines flocculation.
- The solubility of the disperse droplets and the particle size distribution, which in turn determines Ostwald ripening.
- The stability of the liquid film between the droplets, which determines coalescence; and phase inversion (Kabalnov and Shchukin, 1992).

2.1.1. Types of emulsions

Simple or Macro emulsions

Micro emulsions

Multiple emulsions

2.1.1.1. Simple or Macro emulsions

Simple or macro emulsions are also named as conventional emulsions. These are again of two types depending upon the basis of the nature of internal and external phase (Kabalnov and Wennerström, 1996).

2.1.1.1.1. Oil in Water Emulsions

In this type of emulsion continuous phase is aqueous whereas oil constitutes the dispersed phase. Commonly in this type of emulsion, more than 45% of the total weight of the formulation is constituted over the continuous phase. This type of emulsion contains hydrophilic emulsifier. In pharmaceutical field, oil in water type emulsions is more preferred and acceptable than the water in oil type of emulsions, not only for oral use but for cosmetics use also (McAuliffe, 1973). In oil in water emulsion form the medications become much more effective. Because there is good and easy absorption in the body when there is fine and even dispersion of oil globules in water. Moreover water soluble drugs are more rapidly released from oil in water type emulsions and they give positive conductivity test (Viyoch et al., 2013).

2.1.1.1.2. Water in Oil Emulsions

In this type of emulsion the continuous phase is Oil whereas the dispersed or internal phase is water. In this type of emulsions lipophilic emulsifier is used. In cosmetics, they are mostly used in the dry skin treatments as they restrain the evaporation and prevent the dehydration (Opawale and Burgess, 1998). Water in Oil emulsions is cleare and smoother in appearance and this property is attributed to their oily nature. They do not give positive conductivity test (McLean and Kilpatrick, 1997).

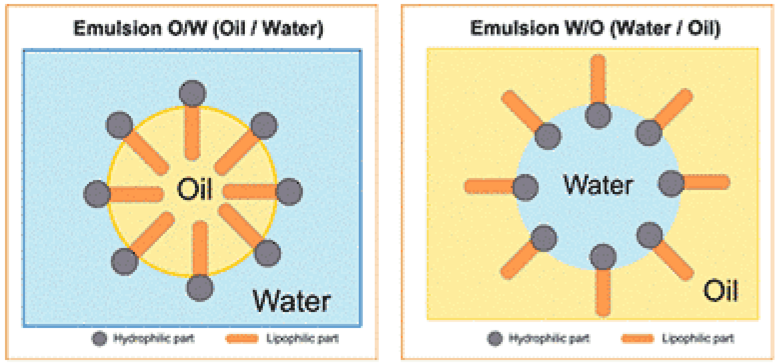


Figure 2.1. Water in Oil and Oil in Water Emulsions

2.1.1.1.3. Multiple Emulsions

These are complex macro systems and referred as double emulsion or emulsion in emulsion because in this type of one simple emulsion is again dispersed in other continuous phase to make multiple emulsions. Multiple emulsions may be classified into two main types; Oil in water in oil multiple emulsions (O/W/O) and water in oil in water multiple emulsions (W/O/W) (Chu et al., 2007).

In an oil in water in oil multiple emulsion (O/W/O), the external continuous phase and the internal droplets are comprises of oil but both are separated from each other by the aqueous phase. Whereas in case of water in oil in water multiple emulsion (W/O/W), the external continuous phase and the internal droplets are composed of water and both of these are separated from each other by the oily phase (Herbert, 1965).

Multiple emulsions are employed to achieve the slow release of the incorporated drug substances. Apart from their advantages and usefulness, multiple emulsions have some limitations also. These limitations and disadvantages are mainly due to their structural complexity and instability (Florence and Whitehill, 1982).

2.1.1.1.4. Micro emulsions

Micro emulsions are defined as ‘these are thermodynamically stable and optically isotopic systems of oil, water and amphiphile’. As they are optically isotopic so they are seem to be clear solutions. They can also be defined as ‘clear dispersion of oil in water or water in oil’. They are identified as true solutions because they are solubilized systems (Schulman et al., 1959). The diameter of the globules of the micro emulsions is very small and ranges from

0.1-1.0 micrometer. This small globular size restricts the light to be refracted through them and they exhibit as true solution. The micro size of the micro emulsion globules represents a very large interfacial area and hence a large free interfacial energy. This makes the micro emulsion a very stable system (Lagües et al., 1978). Micro emulsions have the potential use in the pharmaceutical field as for drug delivery. Lipophilic as well as hydrophilic drugs can be successfully incorporated in them. Micro emulsions give better solubilzation and bioavailability of drugs than the conventional emulsion systems. Other areas of their use are polymer formation from emulsion polymerization, micro encapsulation as well as in cosmetics due to their amazing stability properties (Lagües et al., 1978).

2.1.2. Tests to identify the emulsion system

It is of far most importance to identify the type of prepared emulsion whether the emulsion is oil in water (O/W) or water in oil (W/O) as it strongly affects its performance, quality and stability. Due to the unreliability of the single identification test, the emulsion type must be reconfirmed by a second method. In the emulsion there are two basic constituting components oil and the aqueous phase. Whether the oil or the aqueous phase becomes the external phase depends largely on the concentration of the two components and the type of emulsifier used (Shah, 2007).

Some of the tests to identify the Emulsion system are described below.

2.1.2.1. Dilution Test

The dilution test depends upon the principle that the type of the emulsion can be evaluated with the liquid that is miscible with the continuous phase of the emulsion. It means that the oil in water (O/W) emulsion is diluted with water and water in oil (W/O) emulsion is diluted with oil. If wrong liquid is added the emulsion will break. The results of the test will improve if the addition of the water or oil to the emulsion system is observed with the help of a microscope (Agarwal, 2007).

2.1.2.2. Electrical Conductivity Test

The principle of this test is electric current can pass through water but not through oil. It is done by passing the electric source through the emulsion and connected it to a lamp. The lamp will light if the emulsion is oil in water (O/W) this means that it possess an aqueous external phase. In case of water in oil (W/O) when there is an oily external phase, the lamp will not light (Pearce, 2005).

2.1.2.3. Dye Solubility Test

This test is based on the fact that oil soluble dyes will be dissolved in the oily phase of the emulsion whereas aqueous phase will dissolve the water soluble dyes. So, on microscopic evaluation, if an oil soluble dye is dissolved in the continuous phase of the emulsion, the emulsion will be water in oil (W/O) type. But if the continuous phase does not stained with the dye color then the emulsion will be of oil in water (O/W) type. The test may be repeated by using a water soluble dye (Ho et al., 1996).

2.1.2.4. Cobalt Chloride Test

This is another test for the determination of the emulsion type. In this test a filter paper dipped in an emulsion when dried turns blue to pink in case of oil in water (O/W) type emulsion (Weissenborn and Motiejauskaite, 2000).

2.1.2.5. Fluorescence Test

If the emulsion is exposed to ultra violet radiations and examine microscopically, a dotted fluorescence is observed in case of oil in water (O/W) type emulsion (Porter et al., 1989).

2.1.3. Preparation of Emulsions

At prescription level, for extemporaneous dispensing, simply a pestle-mortar is commonly used for the preparation of emulsions.In recent years many advances have been made not only in the field of pharmaceutical and cosmetic preparation but also on the characteristics of the raw materials. Due to these advancements, at commercial scale, much electrically and manually controlled equipment is being used for emulsion preparation (HERBERT A. L., 1989).

There are various influencing factors that are to be considered while preparing an emulsion, because these affect the overall quality and stability of the final product. These factors include the temperature of oil and aqueous phases, the way of adding the both phases, addition of emulsifier, mixing time and cooling rate after mixing (Menon and Wasan, 1988).

In general, the dispersed phase is changed to the small droplets and then surrounded by the emulsifier and finally these are made to disperse into the continuous phase (JOHN, 1976).

Some commonly used methods are addressed here under:

2.1.3.1. Dry Gum Method (Continental Method)

This method is also called as continental method. In this method simply a pestle-mortar is used for preparing the emulsion. Generally a primary emulsion having water, oil and emulsifier usually acacia is prepared by this method. One part of the emulsifier is taken in the mortar and is triturated with four parts of the oil until a uniform mixture is prepared. Then two parts of water are at once added to the mixture and continuously and strongly triturated until a creamy white colored primary emulsion is formed (Ashok KG, 2008).

2.1.3.2. Wet Gum Method

The proportion of the constituents is same as in dry gum method but method is somewhat different. In this method, one part of the emulsifier is triturated with two parts of water until a uniform mixture is formed. Then the oil is added to it in parts by strong and continuous trituration until a uniform primary emulsion is prepared (Chang and Lindmark, 1986).

2.1.3.3. Bottle Method

Bottle method is used to prepare the emulsion of substances having low densities like volatile oils. In this method four parts of oil are added to a bottle with one part of emulsifier and tightly closed. Then the bottle is shaken very strongly until a uniform primary emulsion is prepared (Waqas et al., 2010).

2.1.4. Processing Equipment for Emulsion Preparation

There are many apparatus which are employed for the preparation of emulsion on small as well as industrial scale. Brief description of some of this equipment is given hereunder:

2.1.4.1. Hand Homogenizer

It is manually operated and controlled apparatus. Coarse emulsions are prepared by this apparatus. The coarse emulsion from the hopper goes through the valve due to the up and down movement of the handle. As a result the size of the oil globules becomes same as that of size of valve (K SURIA P, 2008).

2.1.4.2. Kenwood Mixer

This is a very common apparatus that is used for preparing emulsions on small scale. In this mixer, the beaters are attached to the axis which gives the liquids a rotatory action around the axis (Carter, 2007).

Figure.2.2. Kenwood Mixer

2.1.4.3. Silverson Mixer Homogenizer

It has an emulsifier head having blades on it. This emulsifier head is enfolded within a band of sieve mesh. An electric motor rotates this emulsifier head in the liquid to be emulsified. Meanwhile, the liquid is sucked into the base of the emulsifier head where it is changed into small globules (Lau and Dickinson, 2005).

Figure.2.3. Silverson Mixer Homogenizer

2.1.4.4. Colloidal Mills

These mills are employed for the preparation of the colloidal dispersions. These mills prepare emulsion on continuous bases. The principal of working of these mills is high shear that is produced by the mill stator and rotator (Leon L, 2009).

Figure.2.4. Colloidal Mill

2.1.4.5. Micro fluidizer

Micro fluidizers are used to prepare the emulsion of very small sized globules. The emulsion is subjected to very high velocity and passes through a small orifice which determines the size of the globules. The emulsion is produced by high shear force (RG, 2000).

Figure.2.5. Micro fluidizer

2.1.4.6. Mechanical Stirrers

These stirrers are used for the preparation of emulsions having low viscosity. These are motorized stirrers consisting of impellers attached to a shaft. The speed and rotation of the impeller is adjustable according to the liquids which are to be emulsified (LEON L, 2009b).

Figure.2.6. Mechanical Stirrer

2.1.4.7. Ultrasonifiers

On laboratory scale, homogenization of the emulsions can also be achieved with the help of ultrasonic energy. These ultrasonifiers are extensively used for the preparation of emulsions having low droplet size and moderate viscosity (Leon S, 2004).

2.1.4.8. Turbine Mixers

Turbine mixers are motorized equipment. They are used for the emulsification of those compounds which are very viscous and difficult to mix. In other words, turbine mixers are used when a strong agitation is required (Leon S, 2004).

Figure.2.7. Turbine Mixer

2.1.5. Emulsifier or emulsifying agent

An emulsifier or emulsifying agent is a substance that makes the oil and aqueous phases miscible, reduce the interfacial tension and form a stable emulsion. Emulsifiers are very important and key constituents of an emulsion system (Mahdavian and Sharifi-Sanjani, 2001). They change the characteristics of the particles of the dispersed phase as well as dispersing medium that results in the repulsive and attractive forces. These attractive and repulsive forces between the particles keep the emulsion stabilized. Emulsifiers not only provide stability to the emulsions but also play a role in its texture, structure and functions due to their interactions with other ingredients intra molecularly as well as inter molecularly. The viscosity of an emulsion depends on the concentration of the emulsifier used. Emulsifiers impart long term stability to the formulated emulsion as they decrease the aggregation of the droplets by producing repulsive forces (esoteric and electrostatic) between them (Romero et al., 2009).

Generally, the emulsifiers are molecular surfactants; alcohols, fatty acids, polymers and larger molecular weight proteins like albumin. Structurally they have polar or hydrophilic head and nonpolar tail end. Therefore they are adsorbed to the air oil or air water interface and reduce the interfacial tension (Akhtar et al., 2010b).

There are two types of emulsifier; primary and secondary emulsifiers. Primary emulsifiers are also known as true emulsifiers and they make uniform and stable emulsions. Whereas secondary emulsifiers form the coarse emulsions (Onuki, 1993).

2.1.6. Mechanism of action of an emulsifying Agent

The mechanism of action of an emulsifier depends upon the film which is established at the interface of the two phases. There are three kinds of film are established (B, 2005).

2.1.6.1. Monomolecular Film

Monomolecular type of film is established by the surfactants or surface active agents. They considerably reduce the surface free energy and hence the interfacial tension which ultimately contribute to the stability of the formulated emulsion system. Use of ionic emulsifier adds more to stability due to repulsion of the charged molecules of the established film (Bouchemal et al., 2004).

2.1.6.2. Multi molecular Film

This type of film around the dispersed phase is established mainly by the hydrophilic colloids. When multi molecular film is formed there is no effect on the interfacial tension rather this coating around the dispersed phase avoids coalescence. Moreover these agents increase the viscosity of the emulsion which also contributes to the stability of the emulsion system (Nielloud, 2000).

2.1.6.3. Solid Particle Film

This is type of the film is based on the theory that when a small particle is wetted by both oil and water phase can act itself as an emulsifier (Nielloud, 2000).

2.1.7. Selection of an Emulsifying Agent

The selection of an emulsifying agent is very important in preparing a stable emulsion. Hydrophilic lipophilic balance (HLB system), introduced by Griffin, is a conventional way to select the appropriate emulsifying agent. This system comprises of a scale from 0 to 20. Every emulsifier that exhibits its solubility in oil or water has a specific number. From this HLB system an appropriate emulsifier is chosen for the emulsion which adds to its stability (Mahdavian and Sharifi-Sanjani, 2001).

An ideal emulsifier should have following properties:

- It should be quickly adsorbed at the air water, oil water or air oil interfaces.
- It should reduce the interfacial tension below 10 dyne per cm.
- It should make the stable emulsion at very low concentrations.

- It should improve the emulsion viscosity.
- It should pass some electrical current to produce mutual repulsion.
- It should be non-toxic, non-irritant and suited to the intended use of the preparation.
- It should be compatible with the other ingredients of the emulsion (Carter, 2007).

2.1.7.1. ABIL EM 90

ABIL EM 90 is a silicone based nonionic emulsifying agent. It also functions as emollient and antifoaming agent. It has a chemical name of cetyl dimethicone copolyol. The European Pharmacopoeia 2002 states ABIL EM 90 as a polydimethylsiloxane and is formed by the hydrolysis as well as poly condensation of chlorotrimethylsilane and dichlorodimethylsilane. While the United States Pharmacopeia /National Formulary 20 states ABIL EM 90 as methylated linear polymers of siloxane (Tamburic et al., 1996). It is a colorless, clear, thick liquid and available in many viscosities. Although it is heat stable and resistant to chemicals but is affected by strong acids so it should be kept in air tight containers at a cool and dry place.It is soluble in isopropyl myristate, slightly soluble in ethyl alcohol, miscible with mineral oils, toluene and ethyl acetate whereas insoluble in water, glycerin and propylene glycol (Raymond CR, 2003).

2.1.8. Emulsion Instability

The stability of a prepared emulsion is very important. This stability accounts that it should maintain its real texture and quality; the size of the globules of the dispersed phase should be uniform and they should be evenly distributed throughout the dispersion medium. Moreover, it should not only be chemically stable but should also prevent the microbial growth in it. The stability of the emulsion system depends upon various factors such as nature of the constituents, oil water ratio and nature of the interfacial adsorbed layer. This is also called as oil water interface and it itself depends upon surface charge, concentration of the emulsifier used, hydrophobicity and the competition among various surface active substances present within the emulsion system (Krstonošić et al., 2009).

Emulsions encounter various destabilizing processes like flocculation, creaming, coalescence etc. Identification and control of them is a key aspect of the stability of the commercially prepared emulsions (Boyd et al., 1972).

2.1.8.1. Creaming and Sedimentation

In creaming and sedimentation the globules of the dispersed phase rise up or settle down under the influence of gravity. Creaming and sedimentation can be avoided by mild mixing. The rate of creaming and sedimentation can be minimized when the internal phase is homogeneously dispersed in the external phase, the size of the globules is small, there is less density difference among the particles and emulsion system is more viscous (Robins, 2000).

2.1.8.2. Phase Inversion

This process degrades the emulsion system. In this process oil in water (O/W) emulsion changes to water in oil (W/O) one and vice versa. The most appropriate range for the concentration of the dispersed phase in an emulsion is thought to be 30% to 60% of the total volume. The stability of an emulsion becomes doubtful when the concentration of the dispersed phase exceeds this range. And phase inversion takes place when the concentration of the dispersed phase reaches the 74% of the total volume of the emulsion system (Binks and Lumsdon, 2000).

2.1.8.3. Flocculation

In this process the oil droplets come together and form aggregates but they retain their individualized integrity. This is reversible process and original shape of the emulsion can be regained by a gentle agitation. Sometimes, flocculation is believed to be the precursor of the coalescence (Nambam and Philip, 2012).

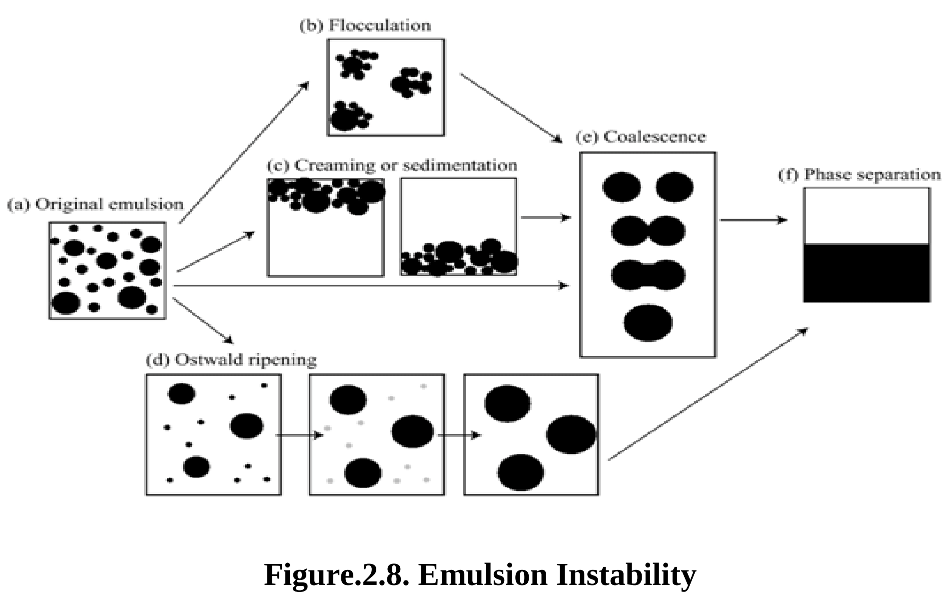


Figure.2.8. Emulsion Instability

2.1.8.4. Coalescence

The most important instability of the emulsion system is that when the emulsion system completely breaks into oil and aqueous phases. This is known as coalescence. This is an irreversible process and regarded as most severe kind of instability of the emulsion system. Irrespective of the mechanism involved, the oil globules tend to accumulate, aggregate become larger in size and eventually join to become a separate oil layer. This is also known as 'Oiling Off' (Boode and Walstra, 1993).

2.1.9. Pharmaceutical and cosmaceutical applications of emulsions

Emulsions have very potential uses in many fields such as pharmaceutical, cosmetics and food. Emulsions has gathered interest of the scientists to be the vehicle for the drug delivery to the body as they have several advantages e.g. enhanced bioavailability of the certain drugs when formulated as emulsion. Furthermore, the therapeutic characteristics and spread ability of the drugs has been found to be increased. In addition to these, emulsion system can also be used for the control release of the drug substances (Bhargava, 1987).

The opportunity of incorporating the incompatible substances into the emulsion systems has opened new gates of research in the field of formulation development. A novel method for surfactant coated enzymes has been formulated by scientists. They used water in oil (W/O) emulsion for their formulation. The surfactant coated enzyme chymotrypsin not only showed high stability but its enzymatic activity also increased remarkably (Goto et al., 1995).

Judicially, Skin protecting preparations are considered to be Cosmetics. In simple words, in order to clarify the difference between the medicinal topical preparations and cosmetics, reference has been made to the legal provisions in the Federal Republic of Germany (Cosmetics Directive, Foods and Drugs Act) (Bleckmann et al., 2006).

Cosmetics can be defined as any article or preparation intended to be used by sprinkling or rubbing to the human body for the purposes of beautifying, promoting attractiveness, changing the appearance, cleaning or improving the skin, hair health. The main purpose of the cosmetics preparations is to restore the skin's natural defense against the environmental factors like UV irradiations and slow down the skin aging process. Recent studies have shown that cosmetics are tending to enhance the positive emotions of the body by affecting the endocrine system, autonomic nervous system and immune systems. Moreover, cosmetics also diminish the levels of stress hormones like cortisol so may elevate the mood (MITSUI, 1998).

Now a days, the important and major use of the emulsions is in cosmetics. Emulsions can easily be formulated, applied topically to the skin to avoid staining and oiliness. Generally, an emulsion is less visible and less noticeable over the skin than a non-emulsified one; this is a major element in the customer acceptance. Emulsion systems for topical application have an added advantage of being emollient. Moreover, emulsions are excellent softener to the skin, good solvent for various drugs and flavors and an inexpensive diluent. Both water in oil (W/O) and oil in water (O/W) type of emulsions is of great importance to the cosmetics industry due to the potential advantage of dissolving the incompatible substances. Hydrophilic active agents like glycolic acid and Vitamin C have shown enhanced stability when formulated as emulsion system for the topical application to the skin. Water sensitive substances such as ascorbic acid have a threat of hydrolysis when in contact with water. It has increased stability when formulated as emulsion (Pössel et al., 2005). Isabelle et al prepared water in oil (W/O) emulsion having ascorbic acid as active ingredient and dimethicone copolyol as an emulsifying agent. This preparation stabilizes the ascorbic acid for topical use in cosmetics. In addition to give stability to the ascorbic acid, this invention also opened the ways of treating the skin conditions like removing the skin pigmentation marks, improving skin complexion, tonifying and smoothing out the skin lines, combating the harmful effects of the UV radiations etc. An oil in water (O/W) emulsion system containing retinoid as active ingredient has good physical as well as chemical stability. This preparation can be employed as an anti-aging preparation because it possess anti-wrinkle properties (Afriat and Chanvin, 2002).

2.2. The Skin

The skin is the largest organ in the human body. Skin is the interface between the internal organs and the environment. In addition to serving as the body’s outermost protective covering, the skin barrier integrates the body’s physiology with the terrestrial environment (Sourla et al., 2000). The thickness of the skin varies between 1.5-4.0mm in different parts of the body and accounts for about 16% of the body weight, has a typical surface area of 1.5 to 2.0 m^2 in adults (Akazaki et al., 2002).

Besides serving as the physical boundary of the body, it has many functions including thermoregulation, physical protection and integrity against dangers from the environment and contact with other objects, synthesis of vitamin D (requiring UV radiation), protection of inner tissues from dehydration and it also acts as a barrier preventing systemic infection from invading surface microorganisms, viruses and allergens (Kankavi, 2006).

2.2.1. Functional anatomy of skin

Skin is a multilayered material with well-defined anatomical regions. Skin is composed of a series of androgen-responsive tissues, namely the hair follicles, sebaceous glands, sweat glands, epidermis and dermis. The skin is a highly organized structure consisting of three main layers, called the epidermis, the dermis and the hypodermis (Martini, 2004).

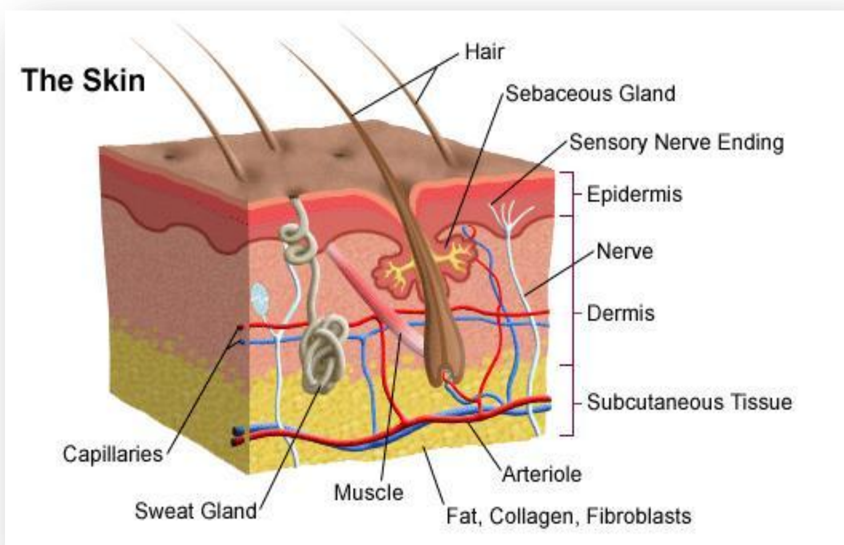


Fig. 2.9. Three main layers of the human skin

2.2.1.1. Epidermis

The skin is the main barrier against organisms and the external environment. Its barrier properties are largely associated with the outermost keratinized layer, the stratum coraeum

(Hemmi et al., 2001). The main functions of epidermis include protection against physical damage, defense against biological invasion, the regulation of the inward and outward passage of materials and the receipt and transmission of signals to other organisms. It serves as an important barrier to the loss of water and other substances of the through the body surface (apart from sweating and sebaceous secretion (Grabe and Neuber, 2005). The epidermis is the most complex structured epithelial tissue. It is an epithelial tissue with a stratified squamous architecture and a cornified surface. As the human epidermis encompasses the whole body, it serves as a signaling interface between the organism and the environment (Jansen and Schalkwijk, 2003).

The epidermis, or top most skin layer, is composed of five different sub layers

1. Stratum corneum
2. Stratum lucidum
3. Stratum granulosum
4. Stratum spinosum
5. Stratum basale

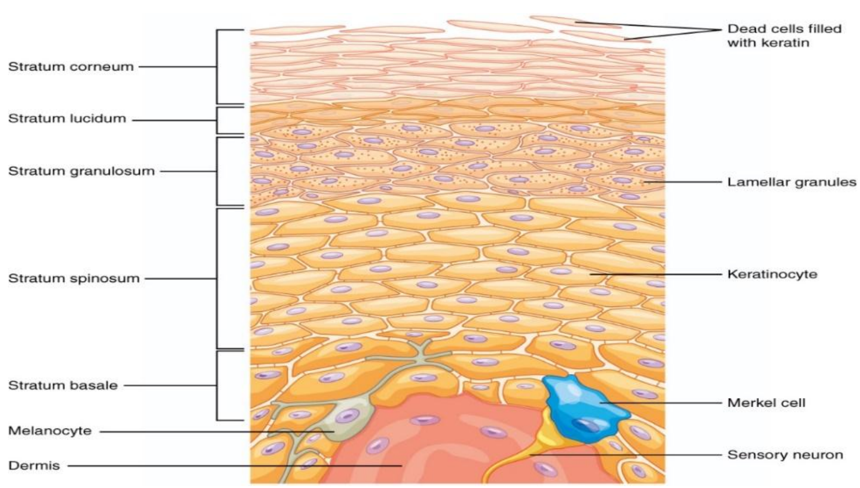


Fig.2.10. Five sub layers of the epidermis

Of these five layers, the stratum corneum is the most impermeable and can be compared structurally to a brick wall. Just as a brick wall consists of bricks and mortar, the stratum corneum consists of flattened cornified cells similar to bricks, which are embedded in a lipid intercellular matrix similar to mortar (Franckum et al., 2003). It consists mainly of one cell type: the keratinocyte, but additional cells are also present; melanocytes producing the skin coloring pigment, immune competent Langerhans cells and neuroendocrine Merkel cells. These are formed in the stratum basale (germinative layer) and gradually migrate upwards to the surface layer — the stratum corneum (horny layer). The stratum corneum contains corneocytes flattened dead cells that have lost their nuclei which are gradually shed. In between the stratum basale and the stratum corneum there is considerable activity, as keratinocytes differentiate and mature into corneocytes. The secretion of extracellular lipids plays an important part in this process. In the stratum granulosum (granular layer), a lipid substance is formed and stored in the lamellar bodies. At the interface between the stratum granulosum and the stratum corneum, lipids are extruded from the cells into the inter-corneocyte space. These “barrier lipids” then form highly organized, multilamellar bilayers. Although cells are continuously shed from the upper surface of the stratum corneum, the deeper layers are firmly bonded together. The integrity of this layer is important as it prevents water loss from the skin. It is held together by the lamellar lipids. The corneocytes themselves contain a water-retaining substance called natural moisturizing factor, which ensures that water is held in the cells. Cells with high water content swell and press tightly against one another, with no cracks or fissures. The healthy stratum corneum has relatively high water content and is elastic and pliable (CLARK, 2004). In addition to the keratinocytes, the basal membrane contains melanocytes, which are cells responsible for pigmenting the skin, with the synthesis of melanin that is progressively transferred to the keratinocytes (Chakraborty et al., 1996).

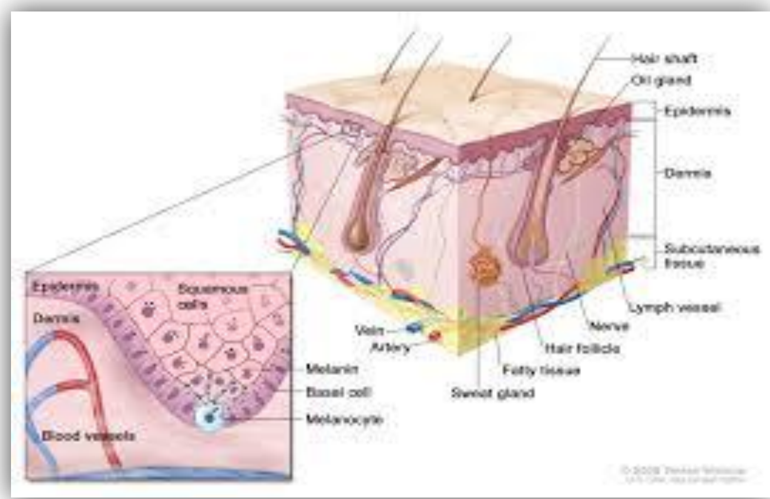


Figure 2.11. Melanocytes (cells responsible for pigmenting the skin)

2.2.1.2. Dermis

The dermis is usually 0.3 to 3 mm thick. It is composed of a dense tissue of collagen and elastic fibers produced by dermal fibroblasts, which provides the physical consistency of the skin**.** The dermis contains blood vessels, hair follicles and sweat glands. The dermis lies between the epidermis and the subcutaneous layer. The dermis has two major components: (1) a superfacial papillary layer and (2) a deeper reticular layer (Silver et al., 2001). The papillary layer consists primarily of small blood vessels and fine collagen and elastic fibers, and the reticular layer contains the larger vascular plexus as well as compact collagen and thick elastic fibers. In both layers, fibroblasts are the primary cell, and they produce much of the extracellular matrix (K, 2006).

2.2.1.3. Hypodermis

The subcutaneous fat or hypodermis is a fibro fatty layer, which is loosely connected to the dermis. Its thickness varies with anatomical site, age, sex, race, endocrine and nutritional status of the individual. It acts as an insulating layer and a protective cushion and constitutes about 10% of the body weight (Hendriks et al., 2006).

Table 2.1. Significance of Epidermal Layers

Epidermal layers	Significance
Basal Layer	Responsible for the production of new cells
	Keratinocytes become corneocytes (dead skin cells) and are subject to desquamation. Responsible for protective functions
Clear layer	Keratinization is achieved by the time when cells have reached the clear layer
	Keratinocytes becomes more granular, less flexible and hardened to complete the keratinization
Spinous layer	This layer consists of living cells which are capable of dividing by mitosis
	This layer is concerned with cell reproduction

2.2.2. Cells in the Skin

2.2.2.1. Melanocytes

Melanocytes are located in the stratum germinativum, squeezed between or deep to the epithelial cells. Melanocytes are responsible for pigmenting the skin, with the synthesis of melanin that is progressively transferred to the keratinocytes (Birbeck et al., 1961). Melanin is responsible for the diversity in human skin colors/tones. Darker skin does not contain more melanocytes; the cells are simply more active. Variation in human skin colour is mainly due to the presence of four pigments, namely, Melanin, Hemoglobin, Carotene and Melanoid.Pigmentation of the skin is controlled by hormones which are synthesized and distributed by the pituitary gland (Mooi and Krausz, 1992).

The metabolic pathway involved in melanin synthesis is extremely complicated involving several intermediate steps. It starts with the amino acid tyrosine oxidized by the copper containing enzyme tyrosinase to dihydroxyphenylalanine (DOPA) and then to dopaquinone. Dopaquinone undergoes a series of non-enzymatic reactions and rearrangements forming the different molecules that are copolymerized to make one of the two types of melanin: Eumelanin, which is the dark brown/ purple/ black compound found in skin/ hair, and Phaeomelanin, which is yellow to reddish brown pigment present in red hair and rarely in human skin. Both forms of melanin combine with other proteins to form the melanosome that is distributed from the melanocytes to the surrounding cells (Okun et al., 1970). Maturation is categorized in four stages: namely stages I and II include unmelanized immature premelanosomes, while melanized melanosomes are classified as stages III and IV. Activation of the melanocortin I receptor promotes the synthesis of eumelanin at the expense of phaeomelanin, although oxidation of tyrosine by tyrosinase is needed for synthesis of both the types of pigments. Both the light and the dark skinned individuals have similar number of Melanocytes for the same body region, but melanosome that contain the pigment are more numerous and more pigmented in darker people than in light skinned people. Melanin biosynthesis can be inhibited by avoiding ultraviolet (UV) exposure, by inhibition of melanocyte metabolism and proliferation. Application of tyrosinase inhibitors may be the least invasive procedure for maintaining skin whiteness; such agents are increasingly used in cosmetic products (Kim et al., 2002).

2.2.2.2. Langerhans Cells

These are present in all layers of epidermis, but are most easily seen in the stratum spinosum; they also occur around blood vessels in the papillary dermis. They have an oval, Reni form or irregular pale nucleus surrounded by pale staining cytoplasm which extends as cytoplasmic processes between the keratinocytes (Barbara Y, 2000).

Ultra structurally, Langerhans cell cytoplasm contains characteristic Birbeck granules, which are rod-like structures with regular cross-striations, one end of which frequently distends in a vesicle so that they resemble a tennis racket. These are antigen-presenting cells and are important component of immune defense mechanism (Merad et al., 2002).

2.2.2.3. Merkel cells

These are specialized touch receptors scattered very sparsely among the cells of the basal layer. By light microscopy they are difficult to distinguish from melanocytes. They contain round neuroendocrine vesicles at their base and have a synaptic junction with a fine nerve ending in the papillary dermis (Moll et al., 1990).

2.2.3. Skin appendages

2.2.3.1. The Sebaceous Glands

Although their size may vary considerably from body region to body region, all mammalian hair follicles have sebaceous glands. Sebaceous glands are alveolar glands attached by a short duct to the opening of primary hair follicles. The sebaceous gland is composed of acini which are attached to a common excretory duct composed of cornifying, stratified squamous epithelium that is continuous with the wall of the piliary canal and, indirectly, with the surface of the epidermis. Sebaceous glands, particularly the very large ones are richly supplied with blood vessels. The glands are numerous on the face and scalp and are sparse in areas such as the back. They can number as high as 400-900 glands per cm2 on the face (Stewart, 1992). Sebaceous glands are usually found in association with a hair follicle, which, together, is referred to as a pilosebaceous unit. These are holocrine glands, forming a secretion by total cellular disintegration. Similar to the colonic mucosa, they contain cells that are constantly going through a process of cell division, differentiation, and cell death. The proliferating sebocytes are situated at the periphery of the gland. As sebocytes move towards the center of the gland they produce lipid and differentiate. Eventually, after some 7-28 days, the cell dissolves, liberating sebum into the sebaceous duct, this directs it to the surface of the skin (Zouboulis, 2004).

2.2.3.2. Sweat Glands

Sweat glands are classified on the basis of their structure and the nature of their secretion. Two types of sweat glands are recognized (Michael HR, 2003):

2.2.3.2.1. Eccrine Sweat Glands

The sweat glands are distributed over the nearly entire body surface except for the lips and part of external genitalia. Eccrine sweat glands have an important thermoregulatory function, but they also respond to emotional stimuli. Under severe heat stress, they are capable of producing up to 10 L of sweat per day, but the normal secretion rate is 0.5–1 mL/min. The total number of sweat glands is somewhere between 2 and 4 million. Only about 5% of the sweat glands are active at the same time, indicating the enormous potential for sweat. The secretory segment of the eccrine sweat gland contains three cells types. These cell types are present in the secretory segment of the glands: clear cells and dark cells, both of which are secretory epithelial cells. And myoepithelial cells which are contractile epithelial cells. All of the cells rest on the basal lamina; their arrangement is that of a pseudo stratified epithelium (Kreyden and Scheidegger, 2004).

2.2.3.2.2. Apocrine Sweat Glands

A different type of sweat gland is found in the axilla and genital region of humans. İn contrast to eccrine sweat gland, these glands are believed to secrete by the apocrine process and are thus called apocrine sweat glands. They also differ in that they produce a viscid secretion which is discharged into the hair follicles rather than directly onto the surface. Apocrine sweat glands are innervated by adrenergic fibers of the sympathetic nervous system. Apocrine sweat glands do not become functional until puberty and in women undergo cyclical changes under the influence of the hormones of the menstrual cycle (Barbara Y, 2000).

2.2.3.2.3. How Sweating Occurs with in the Skin

Sweat is a clear, hypotonic, odorless fluid containing mainly sodium and chloride but also potassium, urea, lactate, amino acids, bicarbonate, and calcium. Proteins, such as immunoglobulin, constitute less than 1% of sweat by weight.

In response to nerve impulses, acetylcholine (ACh) is released from the presynaptic nerve endings and then binds to postsynaptic cholinergic receptors presumably present in the basolateral membrane of the eccrine gland clear cells. Activation of these receptors stimulates an influx of extra cellular Ca into the cytoplasm. The increased intracellular Ca causes a KCl efflux from the cell. Consequently the cell shrinks because water follows the solutes to maintain iso-osmolarity. The decrease in K and Cl leads to a transport of N, K and Cl into the cell in an electrically neutral fashion. The increase in cytoplasmic Na stimulates the Na-pumps to extrude cytoplasmic Na in exchange for extra cellular K, so that in the steady state of secretion K and Na recycle across the basolateral membrane without further loss. In contrast, Cl enters the cell via Na-K-Cl co-transporters and moves into the lumen through the Cl channels at the apical (luminal) membrane. This Cl movement depolarizes the apical membrane and generates the negative luminal potential, which attracts Na into the lumen. Thus, Na and Cl enter the lumen across the cell and join to form NaCl in the isotonic primary fluid. In the oiled portion of the sweat duct, reabsorption of NaCl occurs to preserve electrolytes, creating the hypotonic sweat, which is secreted to the skin surface (Kreyden and Scheidegger, 2004).

2.2.4. Functions of Skin

One of the most striking features of human skin is that it is basically naked; in this way it differs from the skin of most of our warm-blooded relatives. The ancestors of birds and mammals evolved fine thread like appendages on their skin feathers and hairs, respectively, which regulate heat interchange and also help to prevent water loss and mechanical trauma. Lacking such protection, human skin had to undergo numerous structural changes to give it strength, resilience, and sensitivity (J, 2006).

The skin provides;

2.2.4.1. Protection

One of the major functions of healthy skin is the maintenance of physical barrier against the external environment (Tobin, 2006). The skin invests the body to provide a vast physical barrier at the interface with the external environment and is designed to protect us against a range of insults including: desiccant (temperature, electrolyte/fluid balance), mechanical, chemical and microbial. Further protection is provided by the ultraviolet radiation (UVR)-absorbing pigmentation system and the complex immuno-regulatory sentinel networks, which sense (Voegeli, 2009).

2.2.4.2. Heat regulation

Heat transfer from the body core to the skin and from the skin to the environment during heat stress is regulated by thermoregulatory control of skin blood flow and sweating, respectively. Heat loss is facilitated by evaporation of sweat from the skin surface and increased blood flow through rich vascular network of the dermis (Lee and Mack, 2006).

2.2.4.3. Sensation

Skin sensation occurs by way of superficial nerves and their sensory endings. Within the skin are organs with sensory functions: they detect stimuli of touch, pressure, heat, cold and pain

Heat loss is facilitated by evaporation of sweat from the skin surface and increased blood flow through rich vascular network of the dermis (Wang et al., 2007).

2.2.4.4. Synthesis and storage of vitamin D.

The cutaneous generation of vitamin D-3 in humans has been well characterized both in vitro and in vivo. The starting point is the irradiation of a provitamin D, which contains the mandatory D5, 7-conjugated double bonds; in the skin the highest concentrations of 7-dehydrocholesterol are present in the stratum spinosum and stratum basal. After absorption of a quantum of light from sunlight [ultraviolet (UV)-B], the activated molecule can return to its ground state and generate at least six distinct products. The four steroids that do not have a broken carbon bond (the starting 7 dehydrocholesterol and the products pyrocalciferol, lumisterol, and isopyrocalciferol) represent four diastereoisomers with either an a- or b-orientation of the methyl group on C-10 and the hydrogen on C-9. The three secosteroid products all have a broken carbon-carbon bond with a differing position of the three conjugated double bonds; they include previtamin D3, vitamin D3, and tachysterol. In the skin the principal irradiation product produced is previtamin D3, which then undergoes a 1, 7-sigmatropic hydrogen transfer from C-19 to C-9, yielding the final vitamin D3. Under normal sunlight exposure, irrespective of the extent of skin pigmentation, the amount of previtamin D3 produced is not greater than <15% of the available substrate 7-dehydrocholesterol. Further UV exposure simply causes the photoisomerization of previtamin D3 to lumisterol and tachysterol, which are both biologically inert. The newly produced vitamin D3 that is formed in the skin is removed by binding to the plasma transport protein, the vitamin D–binding protein (DBP), present in the capillary bed of the dermis. DBP–vitamin D3 then enters the general circulatory system where it ultimately can be metabolized in the liver to 25-hydroxyvitamin D3 [25(OH) D3, or calcidiol] and then in the kidney to 1a, 25-dihydroxyvitamin D3 [1a, 25(OH) 2D3, or calcitriol] (Holick et al., 2007) .

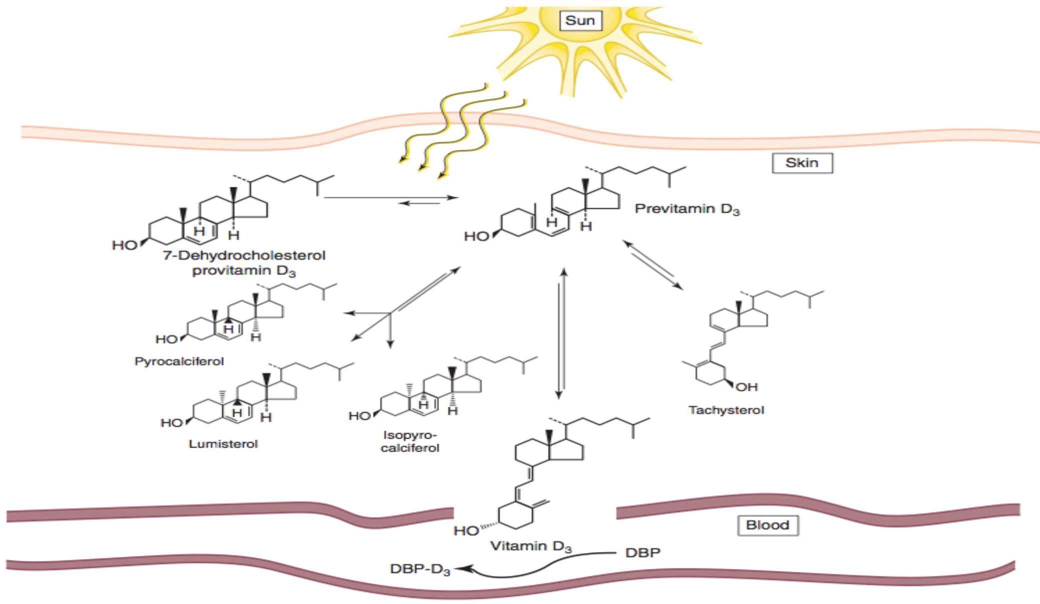


Fig. 2.12. Photochemical pathway occurring in the skin that describes the production of vitamin D3 (cholecalciferol) from 7-dehydrocholesterol.

Vitamin D3 photo production is influenced by season, latitude of residence, and skin color. Wintertime vitamin D3 photo production is compromised in latitudes above 35º. During winter in the higher latitudes, sunlight has a longer tangential path to reach the earth's surface, resulting in the absorption and loss of the UV-B photons in the ozone stratosphere. The role of skin color in vitamin D3 photo production hinges on the function of melanin. Epidermal melanin concentration determines an individual's skin color, and dark-skinned individuals have higher levels of cutaneous melanin. The vitamin D3 precursor, 7-dehydrocholesterol, is predominantly concentrated in the viable deeper layers of the epidermis, namely the stratum spinosum and stratum basale. Melanin acts as a natural filter (sunscreen) and efficiently absorbs the UV-B photons, compromising vitamin D3 photo production in dark skinned people. These individuals need upwards of 6-times greater exposure to UV-B radiation to raise their vitamin D3 levels to the same level as in White individuals (Norman, 1998).

2.2.5. Skin aging

During the natural skin aging process, the collagen content in the dermis decreases approximately 1% per year throughout adult life. Skin aging is a complex process that involves intrinsic and exogenous causes (Fisher et al., 2002).

Extrinsic aging is generally referred to as photo aging and is caused by repeated exposure to ultraviolet (UV) light. Whereas naturally aged skin is smooth, pale, and finely wrinkled, photo aged skin is coarsely wrinkled and associated with dyspigmentation and telangiectasia. Physiologically aged skin is usually characterized by a slow decline in many processes. Alterations in collagen, the major structural component of the skin, have been considered to be a cause of skin aging and are observed in naturally aged and photo aged skin. However, the mechanisms of collagen destruction in aged skin have not been fully clarified (Rittié and Fisher, 2002).

2.2.5.1. Types of aging

2.2.5.1.1. Intrinsic aging

Intrinsic skin aging is associated with other physiological processes and is inevitable. Intrinsic aging is characterized by smooth, dry, pale and finely wrinkled skin.

Generally, the intrinsic skin aging process is characterized by

- Decreased energy levels (ATP) and anabolic processes in the skin cells
- Deficient antioxidant defense mechanisms (genetic polymorphism)
- Deficient melanin synthesis (genetic polymorphism)
- Deficient detox capacity (genetic polymorphism)
- Decreased sexual hormones supply (age-related) and water retention (Uitto, 2008).

2.2.5.1.2. Extrinsic aging

Exogenous aging is caused by extrinsic harmful environments and can be prevented.

Extrinsic skin aging is closely related to

- The photo aging process induced by sunlight or artificial UV-exposure which exerts a major impact on skin appearance through an obvious free radical generation in the skin

- Toxic environmental exposure via smoking, industrial exhausts, heavy metals, detergents, all known as potent free radical inducers
- Chronic infection / inflammatory states associated with an increased free radical attack (superoxide, peroxinitrite, hypochlorite)
- Inappropriate nutrition (excess of refined carbohydrates, fats, food additives, alcohol, low water intake) and last, but not least
- Sleep deficiency and stress.

Ultraviolet (UV) is one of the most noxious factors among the harmful environments. UV irradiation induces inflammatory processes in the skin and the irradiated skin becomes metabolically hyperactive associated with epidermal hyper proliferation and accelerated collagen fiber breakdown (Südel et al., 2005).

Table 2.2. Characteristics of Extrinsic and Intrinsic Skin Aging

Intrinsic Skin Aging	Extrinsic Skin Aging
Very thin skin	Leather-like, thickened appearance
Transparent appearing skin with visible underlying vascular structures	Yellowish discoloration
Fine lines and atrophic crinkling	Deep wrinkles, folds, and furrows
Markedly reduced elasticity and firmness	Reduced elasticity
No pigment changes	Various pigment irregularities
Reduced volume of subcutaneous fat tissue	Reduced volume of subcutaneous fat tissue

2.2.5.2. Factors of aging process

2.2.5.2.1. Free radicals and the aging process

Free radicals are highly reactive chemical species carrying an unpaired electron in their outer orbit. They abstract electrons from the surrounding molecules (lipids, proteins, DNA) in order to complete their own electron structure, thus inducing cellular damage. Such reactions are strongly implicated in the development / pathogenesis of such chronic diseases as atherosclerosis, diabetes, rheumatoid arthritis, cancer, skin and neurodegenerative disorders, as well as in the aging process (Cadenas and Davies, 2000).

To the free-radical family belong Reactive Oxygen Species (ROS) such as superoxide (*O2-), hydrogen peroxide (H2O2), lipid peroxides (LOO*, LO*, LOOH), hydroxyl radical (*OH) and singlet oxygen (Δ-O2); Reactive Nitrogen Species such as nitric oxide (NO*) and peroxinitrite (ONOO*); and Reactive Chlorine Species such as the hypochlorite anion (OCl-). Reactive oxygen species (ROS) are also generated in cell-free systems including O2, H2O2, transition metals as Fe2+/Fe3+, Cu□+/Cu2+, thiols, ascorbate, xanthine/ xanthine oxidase, chelating agents, xenobiotic and others (Ashok and Ali, 1999).

The Free Radical Theory of Aging formulated by Dr. Denham Harmann, postulates that aging is caused by free radical reactions associated with environmental influences, disease, failures of the antioxidative defense and the intrinsic aging process. It predicts that the life span of an organism can be increased by slowing the rate of initiation of free radical reactions and / or decreasing their chain lengths. Following from this theory several mechanisms related to the aging process have been described:

- The endogenic and exogenic free radical generation factors related to disease and aging;
- The pathways of the lipid, protein and DNA oxidative damage induced by the free radical attack along with antioxidant strategies to slow down or block these reactions, as well as the patterns of glycation / oxidation and cross-linking reactions of lipoproteins involved in the aging progression (Harman, 2006).

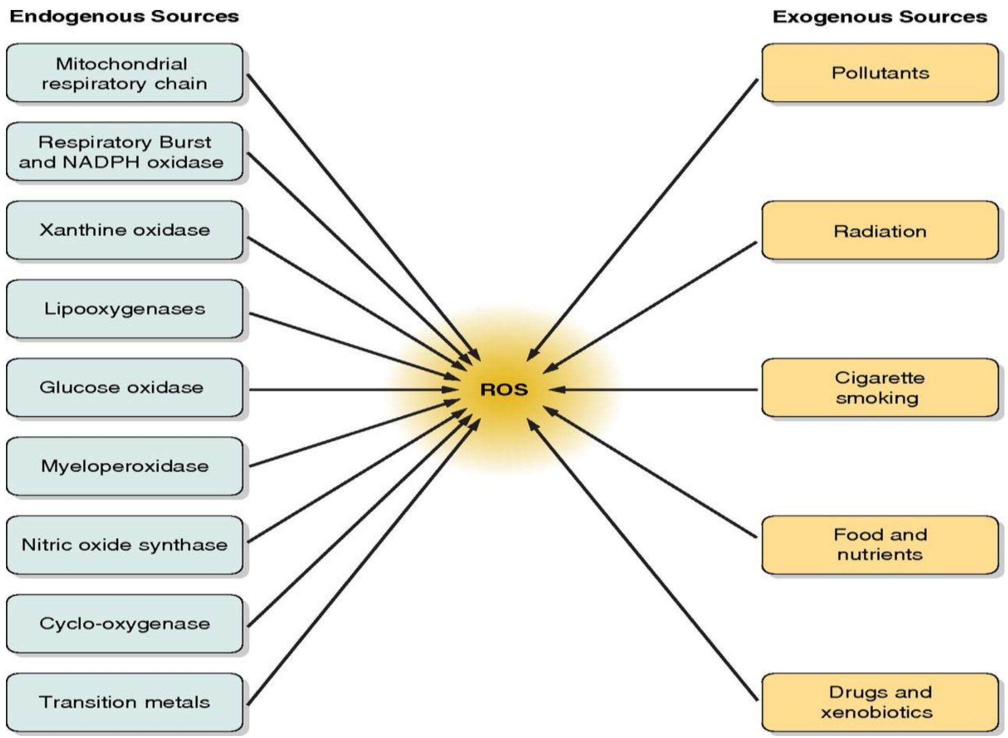


Fig. 2.13. Endogenic and exogenic free-radical triggering factors related to the aging process.

2.2.5.2.2. Sunlight and the aging process

Sunlight is composed of various spectra in frequency of radiation; 50% visible light, 40% infrared and 10% ultraviolet. The UV spectrum consists of 3 main bands UVA (320-400 nm), UVB (290-320 nm) and UVC (<290 nm). Ultraviolet and visible light can induce photochemical changes and significant amounts of UVB can penetrate through the epidermis into the dermis**.** UVC is probably much less carcinogenic than UVB, presumably due to the strong absorption of UVC by the superficial skin layers (Krutmann and Gilchrest, 2006). By producing pyrimidine dimers in DNA chains, UV light penetrating the epidermis can cause significant damage to fundamental processes. The normal responses to sun exposure in the human skin are erythema (largely UVB induced), pigmentation (due to UVA and UVB) and an increase in skin thickness. The chronic changes are - photo-ageing and skin cancer. The commonest form of skin damage due to sunlight is sun bum, the severity of which depends on an endogenous and probably inherited sensitivity of individual skin types to react to UV light. Ageing in the skin is dependent both on the inexorable process of wear and tear as well as other factors, notably sun damage (Fisher, 2005).

Photo ageing is the term used to describe a particular complex of clinico-pathological changes seen in skin which has had long exposure to the sun. Although the terms photo damage, photo ageing, and photo carcinogenesis appeared in the medical literature during the last half century, the fact that sunlight can cause acute and chronic changes in apparently normal skin has been known since antiquity. In addition, the dermis is also altered as shown by the formation of solar elastosis and disorganization of the dermal extracellular matrix occurring during photo aging. Clinically the skin appears coarse, wrinkled, and irregular pigmentation (Fisher et al., 2002). Histologically, the most striking change in photo aged skin is dermal elastosis in which there is an accumulation of thickened elastic fibers and amorphous elastic material in the dermis. A considerable body of evidence is available to support the etiological role of sun exposure in the production of elastosis. The severity of elastosis increases with outdoor occupation, self-reported high lifetime sun exposure and is more severe in people with pale skin than in those with darker skin. Skin taken from areas which are close anatomically and apparently differ only in the amount of sun exposure (e.g. above and below the collar line) shows a consistently greater degree of elastosis in the exposed skin (Yaar and Gilchrest, 2007).

2.2.5.3. Wrinkle

Wrinkles are particularly important signs of advancing age that occur in the direction of the polyhedric mesh structures on the surface of the skin, and can be considered as large skin furrows. Wrinkling and pigmentary changes are directly associated with photo-ageing and are considered its most salient cutaneous manifestations. Such photo damage represents the cutaneous signs of premature ageing. A microscopic look at the surface of the skin reveals a wide variation in its roughness and indentations. The polyhedric mesh structures seen are composed of ridges and furrows, which are commonly referred to as the texture of the skin (Kligman et al., 1985).

2.2.5.3.1. Physiology of wrinkle

Skin changes with age, wrinkles emerge and become more pronounced. Wrinkles depend on nature of skin and muscle contraction. Wrinkles are most important macro structures. Two types of wrinkles are considered: expressive wrinkles (particularly relevant for the face) and wrinkles due to age. Expressive wrinkles also referred to as temporary wrinkles that appear on the face during expressions at all ages and may become permanently visible over time. In addition to their visual effects expressive wrinkles act as an important factor for understanding and interpreting facial expressions, and permanent visible wrinkles indicate the age of a person (Boissieux et al., 2000).

2.2.5.4. Effects of Smoking to Skin

Tobacco smoke is toxic to cells and could be a factor contributing to accelerated skin ageing. Smoking has significant effects on our skin including premature skin ageing, altered wound healing and cancer progression. Epidemiological studies have identified that heavy smokers (defined as those who smoke 1 pack/week) were 4.7 times more likely to have facial wrinkles as opposed to non-smokers, regardless of sun exposure (Kennedy et al., 2003). The thickness of cheek skin is greater in the smokers, but skin thickness does not differ. The amount and width of elastic fibers in the sun-protected skin of the smokers and nonsmokers do not differ significantly, nor do skin elasticity, suggesting that smoking alone affects both the amount and width of dermal elastic fibers or the elasticity of skin in male smokers (Morita, 2007).

2.2.6. Antioxidants

The use of oral and topical antioxidants to provide protection from and treatments for various diseases, including cancer, and to prevent aging, has gained considerable popularity over the past 25 years. While it is clear that antioxidants such as vitamins C, E, A, and carotenoids can protect cells from free radical damage, it is not clear whether taking large quantities of antioxidants can prevent the occurrence of disease or slow the aging process. Some clinical studies have suggested such a role for antioxidants, while other studies have not provided clear evidence that antioxidant supplements can reduce the risk of cancer, heart disease, or aging (Mittler, 2002). Regardless of clinical study results, the fact that antioxidants can block free radical damage to cells has led to rapidly growing markets for antioxidant nutritional supplements, antioxidant beverages, and has fueled an entire industry focused on finding foods with ever - higher antioxidant potential. Most, if not all, topical skincare products manufactured today contain antioxidants such as vitamin E, vitamin C, and carotenoids (Valko et al., 2007).

2.2.6.1. Antioxidants and free radicals

An antioxidant is simply a molecule that can prevent the oxidation of other molecules. Their importance in protecting cells from damage results from their ability to block the progression of oxidative damage caused by free radicals. Free radicals are molecules or atoms with unpaired electrons. Having an unpaired electron leaves an incomplete electron shell and makes the atom or molecule more chemically reactive than those with complete electron shells. Because atoms seek to reach a state of maximum stability, they will try to fill the outer shell by "stealing" an electron from another molecule. When the target molecule loses an electron to the free radical it then, in turn, becomes a free radical and must find a "donor" it can steal an electron from. Thus, a chain reaction begins that cause's considerable damage to cellular proteins, lipids, membranes, and DNA(Fang et al., 2002). Most free radicals in biological systems are derivatives of oxygen. The most common oxygen radicals in the body are the superoxide anion (O2•−) and the hydroxyl radical (iOH). While free radicals have extremely short half - lives, in the order of nanoseconds or microseconds, this is sufficient time to attack molecules and generate new free radicals. In addition to the above oxygen radicals, there are other reactive oxygen species (ROS) which are not actually radicals, but which are reactive and of biological importance. These include hydrogen peroxide and hypochlorite ion. Free radicals can cause extensive and irreversible damage to proteins, DNA, and lipids, and these can have serious consequences on cell survival, malignant transformation, and the development of disease. One of the most damaging free radical events is lipid peroxidation to membrane lipids that have a key role in cell signaling. In this case a hydroxyl radical may remove a hydrogen atom from the side chain of a fatty acid, thereby converting the fatty acid into a radical (Valko et al., 2007). The fatty acid then reacts with oxygen to form a very neureactive peroxyl radical. A chain reaction begins in which one lipid radical becomes two lipid radicals. Ultimately, the lipid radicals may form covalent cross - links with each other thereby ending the chain reaction. Unfortunately, the result is cross - linked and functionally damaged lipids. A good example of this is the free radical damage to low density lipoprotein (LDL). This damage has been shown to lead to atherosclerosis. Antioxidants protect cells from free radical damage by either donating an electron to a free radical thereby stabilizing it and halting the chain reaction or by accepting the one unpaired electron, again stabilizing the free radical and preventing it from interacting with and damaging proteins, DNA, and lipids. By donating an electron to the free radical to stop the chain reaction, the antioxidant itself becomes a free radical (Masaki, 2010). However, because of its structure, the antioxidant is far less reactive than other radicals. If it is relatively large, the effect of the unpaired electron is "diluted" along its structure. The antioxidant "radical" may also be neutralized by another antioxidant or it may be enzymatically restored to its non - free radical form. Glutathione is one antioxidant that can donate a hydrogen atom to a hydroxyl radical thereby neutralizing it. The oxidized glutathione is converted back to its reduced form by glutathione reductase and is then ready to reduce additional free radicals (Calabrese and Maines, 2006).

2.2.6.2. Topical formulations of antioxidants

The development of effective topical formulations that can deliver antioxidants into the skin to protect cells in the epidermis and dermis from free radical attack by UVR, pollutants, and from aging is not a trivial undertaking. Although a number of factors influence the ability of a given molecule to penetrate through the stratum corneum and down to the dermal layer, two important considerations are molecular size and charge. As a rule of thumb, molecules with a molecular weight over 500 Da have a more difficult time penetrating through the stratum corneum along the intercellular lipid route than do smaller molecules. In regard to charge, a molecule that is somewhat hydrophobic has a better chance of penetrating the lipids in the stratum corneum than a water - soluble antioxidant, such as vitamin (Podda and Grundmann-Kollmann, 2001). A variety of antioxidant actives that are used in formulations for skin antiaging include polyphenols of tea extracted from green tea. Reduction in depletion of protective antioxidant enzymes in the skin occur after topical application of green tea extracts which helps in maintaining the levels of both glutathione and glutathione recycling enzymes in UV exposed skin. A potent antioxidant contains polyphenols is extracted from the fruit of coffee plant . Arabica. Grape seed extract is rich in proanthocyanidins flavonoids which are exhibit strong antioxidant activities by scavenging free radicals. The extracts from Pomegranate (*Punica granatum*) can be obtained from various fruit parts as seeds, juice and peel. Phenolic components are included in topical cosmetic formulations for antiaging purposes as they have potent antioxidant activity (Afaq and Mukhtar, 2006). Particularly, UV induced skin damage is shown to decrease in various studies by topical application of fruit extract. Antioxidants deteriorate toxic effects of free radicals that would otherwise destroy skin cells that are healthy and thus they help in protecting the skin, therefore they are important in support to antiaging approaches. The components of the skin antioxidant system that neutralize free radicals become oxidized and inactive. The other components act together in a complex manner as soon as one component becomes inactivated, to restore the antioxidant capability and to recycle the inactive components back to an active state (Cronin and Draelos, 2010). Flavonoids have been reported to exhibit strong antioxidant activities. These are polyphenolic compounds which occur naturally and are universally found in vegetables and fruits of plant kingdom. In many disease conditions such as neurodegenerative disorders, cancer, and cardiovascular diseases, beneficial effects of flavonoids are reported. These compounds possess the iron chelating, free radical scavenging and antioxidant activities and are capable of interacting with the proteins phosphorylation. In conclusion, the incorporation of antioxidants into skincare and topical dermatologic products provides substantial skin benefits. Not only do these compounds protect the skin from free radical damage caused by skin aging and exposure to UVR, but many of them reduce skin inflammation by repressing the activity of inflammatory cytokine and chemokine genes (Rice-evans et al., 1995).

2.3. Literature related to plants used in the study

2.3.1. Soy beans (*Glycine max*)

Soybeans are the seed of the soy plant (Glycine max) is an annual legume of the Leguminosae family that grows to a height from a half meter to one meter. The spheroid seeds, or soybeans, are 8 to 10 mm in diameter and grow within a pod similar to that of peas. It is indigenous to East Asia and China but now is extensively cultivated in many temperate regions of the world. Soy extract has positive research support for its antioxidant, antiproliferative, and anti-carcinogenic activities. This antioxidant, anti-proliferative, anti-angiogenic) extract is used to treat hyperhidrosis in Asian medicine (Jellin et al., 2000). Epidemiologic studies indicating much lower malignancy and cardiac disease rates in people eating a diet high in soy resulted in thorough investigations revealing multiple medicinal uses (Kris-Etherton et al., 2002).

Table 2.3. Taxonomic classification of *Glycine max*

Kingdom:	Plantae
Order:	Fabales
Family:	Fabaceae
Subfamily:	Faboideae
Genus:	Glycine
Species	*G.max*

Topical application of soy has been used to reduce hyperpigmentation, enhance skin elasticity, control oil production, moisturize the skin, and delay hair regrowth. Soy also has the potential to decrease photoaging of the skin and prevent skin cancers through the antioxidant effects of its metabolites (Baumann, 2001). The major components of soy are phospholipids (45–60%), such as phosphatidyl choline, and essential fatty oils (30–35%). The minor components include the most active compounds, such as isoflavones, saponins, essential amino acids, phytosterols, calcium, potassium, iron, and the proteases soybean trypsin inhibitor and Bowman-Birk inhibitor. The most potent isoflavones are the genistein and daidzein (Wei et al., 2003).

Figure 2.14. Seeds of the plant *Glycine max*

Topical application of soy has been used to reduce hyperpigmentation, enhance skin elasticity, control oil production, moisturize the skin, and delay hair regrowth. Soy also has the potential to decrease photo aging of the skin and prevent skin cancers through the antioxidant effects of its metabolites. The major components of soy are phospholipids (45–60%), such as phosphatidyl choline, and essential fatty oils (30–35%). The minor components include the most active compounds, such as isoflavones, saponins, essential amino acids, phytosterols, calcium, potassium, iron, and the proteases soybean trypsin inhibitor and Bowman-Birk inhibitor. The most potent isoflavones are the genistein and daidzein (Wei et al., 2003).

Topical isoflavones have been shown to increase skin thickness and promote collagen synthesis; thus, soy isoflavones stimulation of human fibroblast collagen synthesis is expected. Genistein is the most potent antioxidant; it inhibits lipid peroxidation and chemical and UVB-induced carcinogenesis. Genistein was shown to significantly inhibit chemical, carcinogen induced, reactive oxygen species; oxidative DNA damage; and proto-oncogene expression, as well as the initiation and promotion of skin carcinogenesis in mouse skin. Genistein has been shown to promote collagen synthesis and increase skin thickness, which may be beneficial for postmenopausal women who develop a thinner dermis and decreased collagen (Thornfeldt, 2005). The small proteases STI(Soybean Trypsin Inhibitor) and BBI (Bowman-Birk inhibitor) appear to promote skin lightening and reduce unwanted facial and body hair in human clinical trials The two protease inhibitors lighten pigmented lesions and reduce unwanted facial and body hair in human clinical trials . The small proteases STI and BBI appear to promote skin lightening and reduce unwanted facial and body hair in human clinical trials. Beyond the depigmenting activity, STI, BBI, and soy milk were also found to prevent UV-induced pigmentation both *in vitro* and *in vivo* (Paine et al., 2001). In addition, soy lipids, lecithins, and phytosterols are believed to restore barrier function and replenish moisture (Stallings and Lupo, 2009).

2.3.2. Grape Seed (*Vitis vinifera*)

Vitis vinifera (Family: Vitaceae), is indigenous to southern Europe and Western Asia and is cultivated today in all temperature regions of the world. Parts of this plant are known by several trade names throughout the world: Grape seed extract, grape seed, activin, and others

(Gruenwald et al., 1998). The seeds and the leaves of the grapevine are used in herbal medicine and its fruits are utilized as a dietary supplement (Sato et al., 1999). Grape seed and skin contain several active components including flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidines, and the stilbene derivative resveratrol. Grape seed extract in particular has been reported to possess a broad spectrum of pharmacological and therapeutic effects such as antioxidative, anti-inflammatory, and antimicrobial activities, as well as having cardio protective, hepatoprotective, and neuroprotective effects (Morin et al., 2008).

Table 2.4. Taxonomic classification of *Vitis vinifera*

Kingdom:	Plantae
Order:	Vitales
Family:	Vitaceae
Class:	Magnoliopsida
Genus:	Vitis
Species	*V.vinifera*

Resveratrol is a potent antioxidant that inhibits angiogenesis and carcinogenesis. The sparing/recycling effect of procyanidins from *V. vinifera* seeds on alphatocopherol was established in phosphatidylcholine liposomes and red blood cells. Procyanidines, in addition to scavenging free radicals, strongly and non-competitively inhibit xanthine oxidase activity, the enzyme which triggers the oxy-radical cascade (Baumann, 2004). In one study, polyunsaturated fatty acid peroxidation was inhibited by low concentrations of grape seed proanthocyanidins (2 mg/l)(Bouhamidi et al., 1998). Other studies have confirmed that grape seed proanthocyanidin extract (GSPE) (50 mg/l) provided protection against free radicals in *in vitro* free radical scavenging assay and this effect was better than vitamins C and E (Bagchi et al., 1998). Moreover, GSPE (100 mg/kg), compared to other antioxidants, provided significant protection against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced oxidative damage. In addition, procyanidin B4, catechin, and gallic acid at low concentrations (10 µmol/l, 25 µmol/l) were reported to be good cellular preventive agents against DNA oxidative damage (Simonetti et al., 2002).

Figure 2.15. Seeds of the plant *Vitis vinifera*

However, these compounds may induce cellular DNA damage at higher concentrations (150 µmol/l). Similarly, GSPE demonstrated significant protective ability against oxidative damage in rat leukocytes (Morin et al., 2008).

Recently, co-administration of grape seed extract (75 mg/kg) and *Marjoram volatile* oil (0.16 ml/kg) prevented oxidative damages and resulted in a reduction of the hazardous effects of ethanol toxicity on male fertility, liver, and brain tissues. In this study, rats received ethyl alcohol (10 ml/kg body weight, 25% v/v), daily orally by gavage for 10 weeks (El-Ashmawy et al., 2007). Also, pretreatment with resveratrol (10 µmol) prevented ethanol-induced disruption of embryonic development in blastocysts and ESC-B5 embryonic stem cells(Huang et al., 2007). Resveratrol has also shown protective effects against ischemia reperfusion in the skeletal muscles of rat due to its potent antioxidant properties(Elmali et al., 2007).

Grape seed applied topically improved cutaneous photo protection to UVB, inhibits histamine synthesis, promotes wound healing, reduces apoptosis induced by chemotherapy, reduces vascular engorgement, is cytotoxic to adenocarcinoma, and inhibits Streptococcus (Yamakoshi et al., 2003). Grape seed protects deoxyribonucleic acid (DNA) against oxidation more effectively than vitamins C and E and stabilizes collagen and elastin by inhibiting matrix metalloproteinases. It treats chronic venous insufficiency (CVI) and postoperative edema in clinical studies (Jellin et al., 2000).

All of these functions of grape seed strongly suggest that it should improve photo aged skin and protect against further damage. Grape seed has been used for centuries in Asia to treat a variety of cutaneous conditions.

2.3.3. Tamarind (*Tamarindus indica*)

Tamarindus indica L. belongs to the Leguminosae family (Caesalpinioideae). It is originally from Africa and is grown in humid or arid, tropical and subtropical regions with an average annual temperature of 25 °C; it requires great light intensity and is sensitive to cold. In Brazil, production is located in the North and Northeast (Jindal et al., 2011). The fruit has an elongated pod, 5 to 15 cm long, with dark brown bark; woody and brittle seed in numbers from 3 to 8, surrounded by a brown pulp containing sugar, tartaric, acetic and citric acids (Tsuda et al., 1994).

Table 2.5. Taxonomic classification of *Tamarindus indica*

Kingdom:	Plantae
Order:	Fabales
Family:	Fabaceae
Subfamily:	Caesalpinioideaae
Genus:	Tamaridus
Species	*T.indica*

It is one of the most important fruits used as spice and food source in Africa. Its sweet and sour pulp and fibrous texture is used for preparing sweets, ice cream, liquors, soft drinks and concentrated juices. Practically all parts of the plant are used in folk medicine and it has numerous therapeutic applications in humans, including its usage as digestive, tranquilizer, laxative and expectorant (Komutarin et al., 2004).

Figure 2.16. Seeds of the plant *Tamarindus indica*

Tamarindus indica L. seeds are important sources of antioxidant activity as 2-hydroxy-3', 4'-dihydroxyacetophenone, metdihydroxybenzoate, 3,4dihydroxyphenylacetate and (-)-epicatechin, in addition to oligomeric proanthocyanidins (OPC) (Siddhuraju, 2007). OPCs are potent antioxidant, anti-inflammatory, antihistaminic agent and ultraviolet protection. OPCs also stabilize elastin, collagen and ground substances (Luzia and Jorge, 2011). The detailed study of the composition of *Tamarindus indica* L. seeds will bring contribution to health professionals not only regarding its composition and to evaluate its antioxidant potential, fatty acid profile and content of tocopherols. Biological activity assessment of Tamarind seed was reported on the radical scavenging, lipid peroxidation reducing and anti-microbial activities including anti-inflammatory potential (Lourith et al., 2009).

2.4. Non-invasive bio-physical techniques used in the study

2.4.1. Visioscan VC® 98

SELS is described by four different parameters like roughness (SEr), scaliness (SEsc), smoothness (SEsm) and wrinkling (SEw). Previously profilometry was the only method which is based on skin replica for its calculations to determine the condition of the skin surface. A new method, where the skin can be examined optically using an image-digitalization process without using replica is a big progress in the scientific research. This new method is called surface evaluation of the living skin (SELS). SELS method is determined by Visioscan (Pena Ferreira et al., 2003). The major advantages of Visioscan VC98 are:

1. It is cost effective in comparison to visiometer which utilizes blue dyed silicones

2. Time saving (Primavera and Berardesca, 2005).

Visioscan VC98 is based on a graphic representation of the living skin under special illumination and assessment of this image according to four clinical parameters, SEr, SEsm, SEsc and SEw. The parameters correspond to the condition of skin surface. Visioscan is outfitted with special video camera of UV-A light which captures high resolution (HR) image of the skin and/or hair (Khazaka, 2000).

Fig 2.17. Visioscan VC® 98

2.4.2. Sebumeter®

It is a device by which sebum can be quantified from any area of skin in an objective manner. The sebum measurement on the skin as well as on the hair and scalp is based on the internationally recognized Sebumeter® method. It is a direct measurement of the sebum secretion on skin, hair and scalp. The measurement principle is the photometric method, the grease spot photometer. This method is not sensitive to moisture. The supplied sebumeter-cassette contains a mat synthetic tape which is 0.1 mm thick (Courage and Khazaka, 1988). The measuring head of the cassette exposes a 64 mm^2 measuring section of the tape. For the next measurement the tape has to be transported forward by a trigger at the side of the cassette so that a new measuring section is exposed. One cassette can be used for approx. 450 measurements (if the trigger is only pulled down slowly for the next free measuring section). When completely consumed, the cassette is exchanged for hygienic reasons. A mirror under the measuring section of the tape is protruding approx. 1 mm from the measuring head. This mirror is linked with the cassette by a 0.3 N spring. This makes sure that the tape is pressed onto the measuring area with constant pressure by the mirror. The measuring time of 30 seconds is controlled by a clock in the software. For the determination of the sebum, the measuring head of the cassette is inserted into the aperture of the device, where a photocell measures the transparency. The light transmission represents the sebum content on the surface of the measuring area. A microprocessor calculates the result, which is shown on the display in values from 0-350 (Edwards and Lioy, 2001).

Fig 2.18. Sebumeter (Device & cassette).

2.4.3. Corneometer®

The total amount of water within the *stratum corneum* is of significance both for barrier characteristics and for the clinical manifestation of the skin. This water content can be measured with a new instrument, the CM 825, which takes advantage of the high dielectric constant of water. Corneometer determines the capacitance of the skin as it behaves as a dielectric medium. Corneometer measures a depth of 10-20 µm of the *stratum corneum* of epidermis (Fluhr et al., 1999). A glass lamina in the probe splits the two gold metallic tracks from the skin to avert current conduction in the sample. During measurement of dielectric constant, an electric field is generated. One track of the gold provides negative charge whereas the other provid es positive charge. In this way a complete dielectric constant of water and another substance is measured (Barel and Clarys, 1997).

Figure 2.19. Noninvasive probed Corneometer

2.4.4. Mexameter®

The Mexameter is a skin color-measuring device, and has been reported to provide a reproducible and sensitive means of quantifying small skin color differences. To meet the requirements for a precise determination of the skin color, the Mexameter specifically measures the content of melanin and erythema in the skin. These two components are mainly responsible for the skin color. The measurement is based on the absorption principle. The special probe of the Mexameter emits light of three defined wavelengths(Clarys et al., 2000). A receiver measures the light reflected by the skin. The positions of emitter and receiver guarantee that only diffused and scattered light is measured. As the quantity of emitted light is defined, the quantity of light absorbed by the skin can be calculated. The melanin is measured by two wavelengths. These wavelengths have been chosen in order to achieve different absorption rates by the melanin pigments. For the erythema measurement, two different wavelengths are used to measure the absorption capacity of the skin. One of these wavelengths corresponds with the spectral absorption peak of haemoglobin. The other wavelength has been chosen to avoid other colour influences (e.g. bilirubin). The results for both parameters are shown within 1 second as index numbers between 0 to 999.The same measuring probe is used to quantify both the skin redness (erythema) and determine the degree of skin tanning (melanin). Besides the tanning degree also a decrease in hyper pigmentation due to the effects of bleaching products can be measured. Also irritating effects of cosmetics as well as soothing effects of active agents can be calculated (Baquié and Kasraee, 2014).

Figure 2.20. Mexameter with probe

2.4.5. Skin Elastometer

Elasticity defines the quality of a material (e.g. the skin) to change its shape under application of a force and to return to its original shape when the force is ceased to be applied. To make this clear we could take a balloon and try to change its shape by pressing our finger into the surface. As soon as we stop pressing, the balloon returns completely to its original shape. This means the balloon's skin is 100% elastic. This viscoelasticity of skin surface is determined by the elastin and collagen fibers. In younger skin these fibers are dispersed beneath the skin surface keeping it firm, supple and elastic. With skin ageing, an additional influence like UVA light, mechanical and chemical strain, nicotine, alcohol, genetic predispositions, diseases and many others, the network of fibers gets more stiff and clustered, thus leading to skin slackening and wrinkle formation. The measurement of the skin's viscoelastic properties are therefore a true indicator for the real biological age of the skin. The measurement is the ideal promotion tool for elasticity increasing and anti-ageing products by use of the well-established suction method. Here, the skin's ability to resist pressure (firmness) and its ability to return into the original position when the pressure is released (elasticity) is tested. The results also show pre-mature skin ageing due to lifestyle and sun-exposure. Normally for the measurement 3 seconds suction and 3 seconds relaxation are applied. Elastometer is utilized for the measurement of the skin elasticity. It takes about 6 seconds in measuring the elasticity. The results are shown on the meter as percentage of elasticity (do Prado et al., 2013).

Figure.2.21. Skin Elastometer

CHAPTER 3

MATERIAL AND METHODS

3. MATERIALS AND METHODS

3.1. Chemicals and Apparatus

3.1.1. Chemicals

All chemical used were of analytical grades.

Table 3.1: List of the Chemicals Used	
Chemical	**Manufacturer**
Paraffin oil, Analytical grade Methanol, n-hexane Acetone and Ethanol	Merck KGaA Darmstadt, Germany
Botanical extracts	Department of Pharmacy, IUB, Pakistan
Fragrance	Local Market
Abil EM-90	Franken Chemicals, Gebinde
Distilled Water	Department of pharmacy, IUB, Pakistan

3.1.2. Apparatus and Software

All instruments used were calibrated (where applicable)

Table 3.2: List of the Instruments/Apparatus and Software used

Apparatus	Model or/and Made
Centrifuge machine	Hettich EBA 20, Germany
Cold incubator	Sanyo MIR-153, Japan
Hot incubator	Sanyo MIR-162, Japan
Conductivity-meter	WTW COND-197i, Germany
MPA 5 (Mexameter, Corneometer, Sebumeter)	Courage + Khazaka, Germany
Skin Elastometer	Courage + Khazaka, Germany
Visioscan VC® 98	Courage + Khazaka, Germany
Digital humidity meter	TES Electronic Corp, Taiwan
Electrical balance	Precisa BJ-210, Switzerland
Homogenizer	Euro-Star, IKA D 230, Germany
pH-meter	WTW pH-197i, Germany
Rotary evaporator	Eyela, Co. Ltd. Japan
Dual-beam UV-VIS spectrophotometer	Uvikon XL, Bio-Tek Instruments, Bad Friedrichshall, Germany
DV-III Ultra rheometer	Brookfield Engineering Laboratories
Water bath	HH .$S_{21\ 4.}$ China

Software’s	
Rheocalc	Version 1
SPSS	19.0
Microsoft Office (MS word, MS excel)	2013
EndNote	X7

3.2. Methods

The seeds of grape (*Vitis vinifera*), soybean (*Glycin max*) and tamarind (*Tamarindus indica*) were collected from local market of Bahawalpur, Pakistan. The identification of the seeds was performed at the Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan and a voucher specimen was preserved (voucher # GS-LF-8-15-25, GM-SD-7-16-27, and TI- SD-6-15-87) at the herbarium for future reference.

3.2.1. Extraction Methods

3.2.1.1. Preparation of soy bean seed extract

Plant material (1 g per sample) was ground to a fine powder in a mill and extracted for 20 min with ethanol: hexane 1:1 (50 ml) solvent mixture under sonication in an ultrasonic bath at ambient temperature .The extract residue was removed by filtration through layers of muslin cloth. The extract was concentrated at rotary evaporator. Concentrated extract was filtered through Whatman No.1 filter paper and was stored extract in refrigerator.

3.2.1.2. Preparation of grape seed extract

Grape seed powder (200 g) was extracted with 50% aqueous ethyl alcohol at 60˚C to 70˚C for 2 h. The extract residue was removed by filtration through 16 layers of muslin cloth. The ethyl alcohol was removed under vacuum. The concentrated extract was collected by rotary evaporator and then was filtered through Whatman No.1 filter paper. The extract was stored in refrigerator.

3.2.1.3. Preparation of tamarind seed extract

The crushed seeds were extracted with hexane-ethanol-acetone (50:25:25) for 30 minutes, at a 1:3 seed: solvant ratio, under continuous agitation at room temperature. Then the mixture was filtered through layers of muslin cloth and subjected to rotary evaporation under pressure reduced to 40 °C. The extract was concentrated at rotary evaporator. Concentrated extract was filtered through Whatman No.1 filter paper and was stored extract in refrigerator.

3.2.2. DPPH Preparation and DPPH scavenging activity

1, 1-diphenyl-2-picrylhydrazyl (DPPH) was prepared by taking 1 mg of DPPH in 25 ml methanol to make 100 µl solution. The stable DPPH radical was used for the determination of antioxidant activity. Different concentrations of botanical extracts in respective solvents were added at an equal volume of 10 µl to 90 µl of 100 µM methanolic DPPH solution in a total volume of 100 µl in 96 well plates. The contents were mixed and incubated at 37°C for 30 min. Ascorbic acid was used as standard antioxidant. The reduction in absorbance was measured at 517 nm by using microplate reader; in comparison with the control solution (maximum absorption). The decrease in absorbance indicates increased scavenging activity. The activity is mentioned in percentage form of DPPH radical scavenging according to following formula

Inhibition (%) = [(Absorbance of control - Absorbance of test)/Absorbance of control] × 100

DPPH radical scavenging activity was measured in triplicate.

The overall antioxidant activity of grape seeds (*Vitis vinifera*) was the strongest, followed in descending order by soybean (*Glycin max*) and tamarind (*Tamarindus indica*). The seeds extract of *Vitis vinifera, Glycin max* and *Tamarindus indica* showed 85.61%, 83.45% and 79.26%, DPPH scavenging activity respectively.

3.2.3. Preparation of Cosmetic emulsions containing botanical extracts

3.2.3.1. Preparation of Cosmetic emulsions containing soy bean seed extract

Different formulations by varying the concentrations of Paraffin oil, Abil EM 90 and water were prepared in this study. Oil phase comprised of paraffin oil and surfactant (ABIL- EM 90) heated up to $75^0C \pm 1^0C$. Aqueous phase comprising of water heated to the same temperature and then extract was added in it. In case of base no extract was added in the aqueous phase. W/O emulsions were prepared by adding aqueous phase to the oily phase with continuous stirring at 2000 rpm by the mechanical mixer for 15 minutes until complete aqueous phase added. After complete addition of aqueous phase, the mixer speed reduced to 1000 rpm for homogenization, for 5 minutes, and then the mixer speed further reduced to 500 rpm for a period of 5 minutes for complete homogenization until the emulsion cooled to room temperature. A total of 25 formulations were formed by varying the concentrations of Abil®EM90 and paraffin oil and were kept at 25^oC in incubator for a period of 30 days. After 30 days four formulation were selected to keep them further in different storage conditions (8°C ± 0.1°C, 25°C ± 0.1°C, 40°C ± 0.1°C and 40°C ± 0.1°C) for a period of 21 days. The stable formulation consist of 4% of soy bean seeds extract, 16% paraffin oil, 3% ABIL EM 90, 1% fragrance (Rose oil) and 76% distilled water **(Table. 3.3)** and this stable formulation was selected for further in-vitro study. It was observed for three months at various time intervals with respect to smell and color, type of emulsion, electrical conductivity, centrifugation, liquefaction and the viscosity.

3.2.3.2. Preparation of Cosmetic emulsions containing grape seed extract

Oil phase comprised of paraffin oil and emulsifier (ABIL- EM 90) was heated up to $75^0C \pm 1^0C$. At the same time, aqueous phase comprising of water was also heated to the same temperature. After heating the grape seed extract was added to the heated water. After that, aqueous phase was added to the oil phase drop by drop with continuous stirring at 2000 rpm with the help of mechanical mixer for about 15 minutes until complete aqueous phase was added, 2 to 3 drops of rose oil as fragrant were added during this stirring time. As the aqueous phase completely added, the speed of the mixer was reduced to 1000 rpm for homogenization for a period of 5 minutes. After this the speed of the mixer was further reduced to 500 rpm for 5 minutes for complete homogenization; until the emulsion cooled to room temperature. Base was also prepared by the same method and with same ingredients but without the grape seeds extract.

25 formulations of W/O emulsions were prepared with various concentration of Emulsifier Polysiloxane polyalkyl polyether copolymer (ABIL- EM90), liquid paraffin and distilled water as shown in **(Table. 3.4)**. All these formulations were noted with respect to color, phase separation and liquefaction for 30 days while keeping them at 25°C in incubator. The formulations A2, A16 and A21 were found stable at 25°C. Four samples of each of these three formulations were studied further for 21 days while keeping them further in different storage conditions (8°C ± 0.1°C in refrigerator, 25°C ± 0.1°C, Cold Incubator, Sanyo MIR-153, Japan, 40°C ± 0.1°C, Hot Incubator, Sanyo MIR-162, Japan and 40°C ± 0.1°C, Hot Incubator, Sanyo MIR-162, Japan with 75% relative humidity). The sample A2 was found to be stable at all storage conditions and this sample was selected for further in-vitro study. It was observed for three months at various time intervals with respect to smell and color, type of emulsion, electrical conductivity, centrifugation, liquefaction and the viscosity.

3.2.3.3. Preparation of Cosmetic emulsions containing tamarind seed extract

In this study, W/O emulsion were prepared by adding up of aqueous phase to the oily phase with continuous agitation. Oil phase comprised of paraffin oil and surfactant (ABIL® EM 90) heated up to $75^0C\pm1^0C$. Aqueous phase comprising of water heated to the same temperature and then extract was added in it. In case of base no extract was added in the aqueous phase. After that, aqueous phase added to the oily phase drop by drop with constant stirring at 2000 rpm by the mechanical mixer for 15 minutes until complete aqueous phase added. After complete addition of aqueous phase, the mixer speed was reduced to 1000 rpm for homogenization, for 5 minutes, and then the mixer speed further reduced to 500 rpm for a period of 5 minutes for complete homogenization until the emulsion cooled to room temperature (shown in Figure.1). 25 formulations of W/O emulsions were prepared with various concentration of Emulsifier Polysiloxane polyalkyl polyether copolymer (ABIL-EM90), liquid paraffin and distilled water. All these formulations were noted with respect to color, phase separation and liquefaction for 30 days while keeping them at 25°C in incubator. The formulations F7, F16 and F21 were found stable at 25°C. Four samples of each of these three formulations were studied further for 21 days while keeping them at 8°C, 25°C, 40°C, and 40°C +75% RH. The sample F16 was found to be stable at all storage conditions as shown in **(Table. 3.5)** and this sample was selected for further in-vitro study. Both formulation and base were evaluated for three months with respect to smell and color, type of emulsion and electrical conductivity, centrifugation, liquefaction and the viscosity.

Table.3.3. The composition of stable Base & Formulation (Soybean seeds)

Cosmetic Emulsion	Paraffin oil (%)	Abil EM 90 (%)	Soybean seeds extract (%)	Fragrance (Rose oil) (%)	Distilled water (%)
Base	16	3	Nil	1	q.s. 100%
Stable Formulation (E1)	16	3	4	1	q.s. 100%

Table.3.4. The composition of stable Base & Formulation (Grape seeds)

Cosmetic Emulsion	Paraffin oil (%)	Abil EM 90 (%)	Grape seeds extract (%)	Fragrance (Rose oil) (%)	Distilled water (%)
Base	16	4	Nil	1	q.s. 100%
Stable Formulation (A2)	16	4	4	1	q.s. 100%

Table.3.5. The composition of stable Base & Formulation (Tamarind seeds)

Cosmetic Emulsion	Paraffin oil (%)	Abil EM 90 (%)	Soybean seeds extract (%)	Fragrance (Rose oil) (%)	Distilled water (%)
Base	14	2.5	Nil	1	q.s. 100%
Stable Formulation (F16)	14	2.5	4	1	q.s. 100%

3.2.4. Properties of cosmetic emulsions

Stability of emulsions was analyzed to assure the formulation of desired emulsion.

3.2.4.1. Physical analysis

Organoleptic (color, thickness, appearance, feel) and physically (creaming and phase separation) evaluation of emulsion were analyzed.

3.2.4.2. Types of emulsions

Type of emulsions was analyzed by diluting the emulsion with oil and water separately. .

3.2.4.3. Centrifugation tests

After preparation, Centrifugal tests were performed for cream immediately. The centrifugal tests were repeated for emulsion after 12hours, 24 hours, 48 hours, 7 days, 14 days, 21 days, 28 days, 60 days and 90 days of preparation. The centrifugal tests were performed at 25 ± 0.5^0C and at 5000 rpm for 10 minutes by placing the 5g of sample in disposable stoppered centrifugal tubes.

3.2.4.4. Stability tests

At different temperatures, Stability tests were performed for emulsions to note the effect of these conditions on the storage of emulsions. These tests were performed on samples kept at 8^0C $\pm 0.5^0$C (in refrigerator), 25^0C $\pm 0.5^0$C (in incubator), 40^0C $\pm 0.5^0$C (in incubator) and 40^0C $\pm 0.5^0$C (in incubator) & 75% relative humidity (RH). Physical characteristic of simple emulsions, i.e. color, creaming and liquefaction, were noted at various intervals for 12 weeks.

3.2.4.5. Rheological tests

Rheological test of stable creams having different constituents were performed for freshly prepared base and active formulations stored at different conditions in order to see any change produced by these conditions. For this purpose, programmable cone plate (Brookfield) rheometer was used. Approximately 0.2 g samples and constant temperature of 25 ± 0.5^0C were used for the tests. Tests were repeated three times, each containing 9 values of share rate. Flow curve were fit to one of the available mathematical model. These tests were performed by using 0.2 g of creams at 25 ± 0.5^0C. Increased shear stress was applied and shear rate and changes in viscosity were observed

3.3. Study protocol:

The study was designed single blinded –placebo controlled for the comparisons of two emulsions in which volunteers were not informed about the ingredients in the formulation. After obtaining written informed consent **(Table. 3.6)**, selected cosmetic emulsion formulations and their respective bases were handed over to thirty three (11 for each active formulation) healthy male human volunteers whose ages were in between 25 and 35 years for application on respective cheeks for a duration of twelve weeks. Prior to the tests, a cosmetic expert for any serious skin disease or damage especially on cheeks and forearms examined the volunteers. On the first day, patch test (Burchard test) was performed on the forearms of each volunteer to determine any possible reactions to the emulsions. On the second day, each volunteer was provided with two emulsions (creams). One emulsion (cream) was base and the other one was formulation containing the active ingredients. Each emulsion (cream) was marked with "right" or "left" indicating application of that emulsion (cream) to the respective cheek. The volunteers themselves as instructed applied the emulsions (creams) for 12 weeks. Values for different parameters were taken in controlled room temperature 25 ± 1°C and 45 ± 2 % relative humidity. The site of measurement was the whole cheek. Every volunteer was instructed to come for measurement on 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th} and 12^{th} week. Values for each parameter was measured three times (n=3).

<table>
<tr><th colspan="6">Table 3.6: Volunteer Protocol for Written Consent</th></tr>
<tr><td>Study level</td><td colspan="5">Ph.D.</td></tr>
<tr><td>Institute</td><td colspan="5">Faculty of pharmacy & alternative medicine</td></tr>
<tr><td>University</td><td colspan="5">The Islamia university of Bahawalpur.</td></tr>
<tr><th colspan="2">Researcher</th><th colspan="2">Volunteer</th><th colspan="2">Supervisor</th></tr>
<tr><td>Name</td><td></td><td>Name</td><td></td><td>Name</td><td></td></tr>
<tr><td rowspan="3">Signature/Date</td><td rowspan="3"></td><td>Age</td><td></td><td>Designation</td><td></td></tr>
<tr><td>Gender</td><td></td><td rowspan="2">Signature</td><td rowspan="2"></td></tr>
<tr><td>Signature/Date</td><td></td></tr>
</table>

3.3.1. Patch Test (Burchard Test)

On the first day of skin testing, patch tests were performed on the forearms of each volunteer. A 5cm X 4cm region was marked on both the forearms. Basic values for erythema and melanin 0okl.were measured with the help of Mexameter. 1.0 g of base and formulation each were ap[plied to the 5cm X 4cm marked regions separately on each forearm. The regions were covered with the surgical dressing after application. After 48 hours, dressings were removed and the measurements of erythema and melanin were repeated on both forearms.

3.3.1.1. Essential criteria for being included in the study

1) Individuals should be healthy male or female.
2) No person should be hypersensitive to creams. There should be no lesions, diseases or any abnormality on the skin of face and forearm.
3) Nobody should use any other cream or any other product on the face, at least 3 days before the study.
4) Every individual should read and sign the volunteer protocol.
5) During the study, every individual should come to the laboratory for measurements.

3.3.1.2.Criteria for Excluding Volunteers from the study

1) Pregnant women or milk feeding women.
2) Any individual under treatment for skin disorders during the study.
3) Any individual hypersensitive to any ingredient of the cream or with history of sensitivity.
4) Any woman taking estrogen, progesterone or any other oral contraceptive at least 12 weeks before the study.
5) Any individual who does not obey the application rules of the creams.
6) Any individual with extraordinary hair on the cheeks.

Nobody can insist to be informed about the composition of emulsions (creams) or about the results of the study.

3.3.2. Non-invasive *In-Vivo* Evaluation

3.3.2.1. Skin Melanin Contents

Mexameter® was used to measure melanin on the cheeks. melanin contents of the volunteers were measured at the start of the study (zero hour), before the application of the emulsions (creams) and then on week 2, week 4, week 6, week 8, week 10 and week 12.

3.3.2.2. Skin Sebum Contents

Sebumeter was used to determine sebum content of the skin before application of any emulsion (cream) and then on week 2, week 4, week 6, week 8, week 10 and week 12 by using Sebumeter®.

3.3.2.3. *Stratum Corneum* Water Contents

With the help of a Corneometer®, *stratum corneum* water contents were measured before the application of any emulsion (cream) and then on week 2, week 4, week 6, week 8, week 10 and week 12.

3.3.2.4. SELS Determination by Visioscan

Four major SELS (SE*r*, SE*sc*, SE*sm* and Se*w)* parameters were measured at baseline (0 time values) and then at 1st, 2nd and 3rd month of study period by Visioscan® VC 98.

3.3.2.5. Skin Elasticity

Skin Elasticity values of the volunteers were measured on the cheeks at the start of the study (zero hour), before the application of the emulsions(creams) and then on week 2, week 4, week 6, week 8, week 10 and week 12 by using Skin Elastometer®.

3.3.3. Ethical standards

This work was authorized by the Board of Advanced Studies and Research (BASR), and its Ethical Committee for in vivo Studies (Reference No. 4710/Acad.), The Islamia University of Bahawalpur, Pakistan and was directed according to the international guidelines of Helsinki Declaration.

3.3.4. Panel Test

Every individual was provided with a form prepared previously to test the sensory values of cream. This form consisted of seven parameters to be evaluated and every parameter was assigned 11 values from –5 to +5 indicating very bad to very good, respectively **(Figure 3.1.)**. This form was asked to be completed independently by each individual on week 12.

3.3.4.1. Panel Test Parameters

1. Ease of application
2. Spreadibility
3. Sense just after application
4. Sense in long-term
5. Irritation
6. Shine on skin
7. Sense of softness

Figure 3.1. Form of the Panel Test

3.4. Mathematical Analysis

The percentage changes for the individual values of different parameters of volunteers taken at different intervals were calculated by the following formula;

Percentage Change = [(A – B) / B]*100

Here;

A = Individual value of any parameter of 2nd, 4th, 6th, 8th, 10th and 12th week.

B = Zero hour value of that parameter

3.5. Statistical analysis:

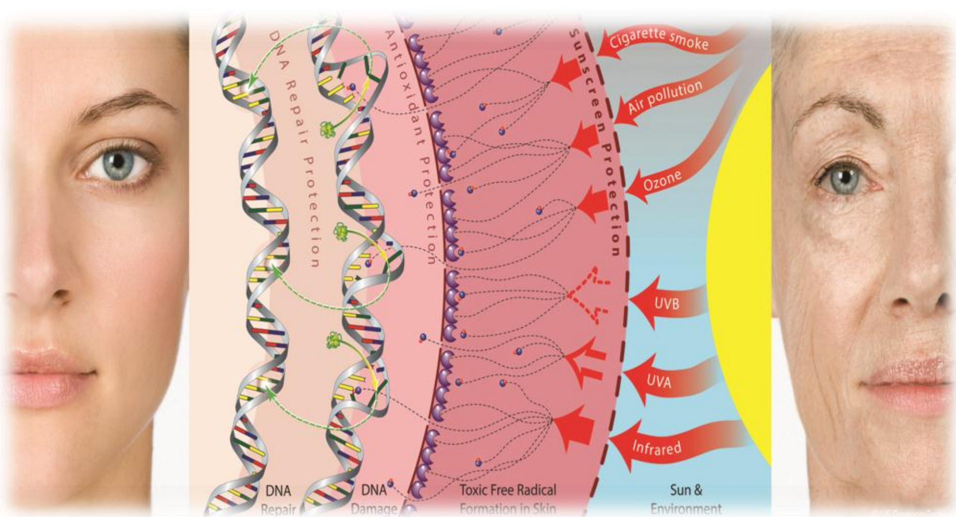
The percentage changes with respect to initial values/zero hour of volunteers for different parameters, taken at 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th} or 12^{th} week were calculated. The measured values obtained for different parameters (skin moisture, sebum, melanin, erythema, elasticity and SELS) were analyzed using SPSS 19.0 on the computer. The tools applied were

- Paired samples t-test for variation between the two preparations.
- Two-way ANOVA for variation between different time intervals.

The level of significance was established at 5% ($p = 0.05$).

CHAPTER 4

RESULT AND DISSCUSSION



4. Results and discussion

4.1. Results

4.1.1. Antioxidant activity of botanical extracts

Botanical extracts of seeds of the research plants (*Vitis vinifera*, *Tamarindus indica* and *Glycin max)* were screened for their free radical scavenging properties using ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The overall antioxidant activity of grape seeds (*Vitis vinifera*) was the strongest, followed in descending order by soybean (*Glycin max*) and tamarind (*Tamarindus indica*). The seeds extract of *Vitis vinifera, Glycin max* and *Tamarindus indica* showed 85.61%, 83.45% and 79.26%, DPPH scavenging activity respectively.

4.1.2. Stability Testing of Emulsions containing various botanical extracts

To evaluate the stability of both the base and formulation (containing different botanical extracts), they were subjected to varying conditions of temperature and humidity. Both base and formulation were divided into four portions and these samples were then kept in refrigerator at 8±^{0} C and in incubator at 25±0.5^0 C, 40±0.5^0 C and 40±0.5^0 C+75% Relative Humidity. These samples were studied for physical characteristics like Color, Phase separation and Liquefaction for 90 days period. **(Table. 4.1)**, **(Table. 4.2)**, **(Table. 4.3)**

Table 4.1. Physical Charactristics of Soybean Base (SB) and Soybean Formulation (SF) Kept 8± 0.5°C, 25± 0.5 °C, 40± 0.5 °C and 40± 0.5 °C +75%RH

		Fresh		After 12 hrs		After 24 hrs		After 48 hrs		After 7 days		After 14 days		After 21 days		After 28 days		After 60 days		After 90 days	
		B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F
Color	A	W	PY	W	PY	W	PY	W	PY	W	PY	W	PY	W	PY	W	PY	w	PY	W	PY
	B	W	PY	W	PY	W	PY	W	PY	W	PY	W	PY	W	PY	W	PY	w	PY	W	PY
	C	W	PY	W	PY	W	PY	W	PY	W	PY	W	PY	W	PY	W	PY	w	PY	W	PY
	D	W	PY	W	PY	W	PY	W	PY	W	PY	w	PY	W	PY	W	PY	w	PY	W	PY
Liquefaction	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
	D	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
Phase Separation	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
	D	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**+ve**	**-ve**

W=White, PY=Pale Yellow -ve =No change, +ve = little change; A=At 8± 0.5^{0}C; B=At 25± 0.5^{0}C; C= At 40± 0.5^{0}C; D= At 40^{0}± 0.5°C+75%RH(Relative Humidity)

Table 4.2. Physical Charactristics of Grape seeds Base (GB) and Grape seeds Formulation (GF) Kept 8± 0.5°C, 25± 0.5 °C, 40± 0.5 °C and 40± 0.5 °C +75%RH

		Fresh		After 12 hrs		After 24 hrs		After 48 hrs		After 7 days		After 14 days		After 21 days		After 28 days		After 60 days		After 90 days	
		B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F
Color	A	W	LP	w	LP	w	LP	W	LP	W	LP	W	LP	W	LP	W	LP	w	LP	W	LP
	B	W	LP	w	LP	w	LP	W	LP	W	LP	W	LP	W	LP	W	LP	w	LP	W	LP
	C	W	LP	w	LP	w	LP	W	LP	W	LP	W	LP	W	LP	W	LP	w	LP	W	LP
	D	W	LP	w	LP	w	LP	W	LP	W	LP	W	LP	W	LP	W	LP	w	LP	W	LP
Liquefaction	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
	D	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve
Phase Separation	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
	D	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**+ve**	**-ve**

W=White, LP= Light Pink -ve =No change, +ve = little change; A=At 8± 0.5^{0}C; B=At 25± 0.5^{0}C; C= At 40± 0.5^{0}C; D= At 40^{0}± 0.5°C+75%RH(Relative Humidity).

Table 4.3. Physical Charactristics of Tamarind seeds Base (TB) and Tamarind seeds Formulation (TF) Kept 8± 0.5^{o}C, 25± 0.5 ^{o}C, 40± 0.5 ^{o}C and 40± 0.5 ^{o}C +75%RH

		Fresh		After 12 hrs		After 24 hrs		After 48 hrs		After 7 days		After 14 days		After 21 days		After 28 days		After 60 days		After 90 days	
		B	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**
Color	**A**	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P
	B	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P
	C	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P
	D	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P	W	P
Liquefaction	**A**	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
	D	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve
Phase Separation	**A**	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
	D	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**+ve**	**-ve**

W=White, P=Pinkish, -ve =No change, +ve = little change; A=At 8± 0.5^{0}C; B=At 25± 0.5^{0}C; C= At 40± 0.5^{0}C; D= At 40^{0}± 0.5^{o}C+75%RH (Relative Humidity).

4.1.3. Determination of type of emulsion

The drop dilution method is adopted for detection of emulsion type. A certain amount of emulsion was taken in test tube and diluted with certain amount of water. The emulsion was immiscible and did not dilute with water confirming this emulsion was w/o type.

4.1.4. Centrifugation Tests

Centrifugation tests for all bases and formulations kept at different storage conditions were performed and phase separation in samples kept at different storage conditions was observed for 90 days at different time intervals. No phase separation after centrifugation was found in any of the samples of base and formulation.

4.1.5. Rheological evaluations

Rheological characteristics of emulsions kept at different storage conditions up to three months were evaluated. Rheological properties of emulsions containing soybean seeds extract are given in **(Table. 4.4-4.8)** while Rheogram of emulsions containing soyabean seeds have been presented in figures **(Figure. 4.1-4.14).** Rheological properties of emulsions containing grape seeds extract are given in **(Table. 4.9-4.13)** while Rheogram of emulsions containing grape seeds have been presented in figures **(Figure 4.15-4.28).** Rheological properties of emulsions containing tamarind seeds extract are given in **(Table. 4.14-4.18)** while Rheogram of emulsions containing tamarind seeds have been presented in figures **(Figure 4.29-4.42).**

Table 4.4. Viscosities (cP) of SB and SF kept at 8 ±0.5ºC

Sr.No	Zero hour		15 days		30 days		45 days		60 days		75 days		90 days	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	10288.13	10569.61	8299.51	8349.991	5846.2	9478.3	6651.544	8659.324	6474.712	8344.955	6405.944	8021.523	6012.984	7581.367
2	9505.110	9770.663	7891.823	7636.523	6534.883	9345.775	6349.456	8446.008	6077.195	7659.731	6145.608	7643.767	5733.734	7288.344
3	9110.225	9198.643	7528.335	7318.471	6371.55	9092.807	5988.424	8071.468	5717.568	7100.991	5723.176	7450.971	5521.783	6911.347
4	8510.810	8668.147	7207.827	7015.155	6187.35	8839.83	5640.9	7755.516	5282.32	6651.543	5304.428	7240.515	5277.411	6439.263
5	8354.821	8257.995	6933.983	6657.807	6017.895	8448.107	5112.327	7418.172	5015.847	6286.827	5146.016	6824.223	5060.815	6312.843
6	8042.865	7871.175	67854.92	6640.615	5852.115	8182.85	5044.091	7004.336	4765.335	5982.283	4761.652	6552.835	4887.667	6136.011
7	7678.847	7523.651	6567.570	6181.343	5705.983	8002.343	4652.35	6644.176	4555.347	5714.57	4490.264	6282.675	4631.014	5860.711
8	7519.73	7216.651	6367.404	5413.843	5559.851	7640.083	4374.590	6137.012	4361.323	5541.611	4244.664	5782.87	4276.591	5644.583
9	7288.875	6928.071	6190.574	5241.923	5412.491	7285.191	3921.168	5768.612	4189.403	5242.155	4013.8	5518.85	4021.167	5391.615
10	7074.201	6561.594	5914.969	5527.511	5188.462	7097.534	3404.341	5196.526	4034.673	4825.330	3907.190	5047.775	3913.815	5028.127

Table 4.5. Viscosities (cP) of SB and SF kept at 25±0.5ºC

Sr.No	Zero hour		15 days		30 days		45 days		60 days		75days		90 days	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	9199.22	9970.61	7980.22	8416.874	6549.622	9140.8	6444.013	8473.898	5851.40	6962.923	6825.755	6235.252	5705.84	5930.709s
2	9606.110	9770.663	7431.322	8012.330	6211.90	8524.245	6238.937	8170.59	5291.450	6412.083	6443.847	5914.744	5584.248	5686.337
3	9111.222	9199.643	7038.362	7426.042	5866.850	8491.089	5816.505	7936.034	4925.505	6044.911	5854.571	6028.948	5500.746	5453.017
4	8715.810	8669.147	6817.322	7177.290	5518.645	8001.117	5670.373	7706.398	4639.382	5699.843	5629.847	5411.264	5271.108	5207.417
5	8365.830	8258.995	6500.4	6827.310	5208.642	7450.973	5223.39	7288.878	4406.062	5403.894	5403.895	5163.044	5161.818	5014.63
6	8042.866	7872.175	6205.76	6331.0	5041.633	7227.477	5151.993	7070.294	4202.214	5368.11	4993.743	4863.412	5039.018	4716.217
7	7778.846	7524.651	5834.922	6382.444	4789.894	6898.373	4600.785	6557.686	4033.96	5083.387	4603.23	4587.112	4902.708	4539.385
8	7519.72	7217.651	5730.542	5982.94	4470.613	6926.617	4390.797	6302.28	3885.390	5102.871	4344.131	4370.984	4821.68	4216.43
9	7288.874	6929.071	5519.326	5524.400	4304.833	6682.245	4281.35	6024.734	3769.94	5118.835	4213.963	3995.216	4674.5	4073.973
10	7075.202	6662.595	4970.410	5447.732	3763.285	6203.325	4351.337	5748.434	3634.86	5116.37	4091.163	3877.328	4588.36	3941.349

Table 4.6. Viscosities (cP) of SB and SF kept at 40±0.5C º

Sr.No	Zero hour		15 days		30 days		45 days		60 days		75days		90 days	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	12298.22	11570.64	8247.77	9518.77	6681.016	7997.557	5163.332	7984.988	6257.352	6883.471	6811.0231	5484.248	6275.612	5302.35
2	9906.111	9970.663	7825.511	9221.585	6462.432	7803.533	4861.244	7723.424	6082.93	6690.675	6200.703	5165.516	6144.216	5043.22
3	9411.2282	9399.643	7420.271	8820.029	6145.444	7550.565	4457.232	7329.236	5667.914	6249.823	5793.007	4595.872	5887.564	4856.57
4	8915.811	8869.147	7104.675	8414.789	5912.124	7209.19	4088.832	6821.54	5352.34	5874.055	5273.563	4802.176	5161.816	4552.031
5	8565.831	8458.995	6857.847	8156.909	5357.232	6864.113	3711.836	6723.832	5060.055	5735.291	5431.975	4428.864	4823.053	4725.17
6	8242.862	7872.175	6195.955	7709.917	5088.303	6749.909	3536.232	6473.32	4836.51	5176.551	5418.467	4163.616	4916.216	4343.271
7	7978.842	7724.651	5746.507	7337.833	4902.706	6408.525	3182.568	5937.912	4641.302	4779.907	5368.11	3795.216	4926.737	3815.231
8	7719.72	7417.651	5309.33	6809.957	4682.894	6165.371	3004.508	5655.472	4468.13	4546.587	5253.915	3413.308	4835.333	3589.27
9	7488.872	7129.071	5253.915	6543.49	4486.414	6052.403	2771.188	5370.576	4298.694	4288.707	5118.835	3113.676	4810.773	3309.295
10	7275.202	6862.595	4579.907	6305.249	4175.732	5707.335	2637.336	4779.908	4169.755	4180.643	4981.306	2788.256	4781.389	2891.93

Table 4.7. Viscosities (cP) of SB and SF kept at 40±0.5ºC+ 75%RH

Sr.No	Zero hour		15 days		30 days		45 days		60 days		75days		90 days	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	9299.23	11570.63	7408.524	8358.426	6528.744	9300.178	6206.843	7942.008	5792.476	6192.109	6254.737	5869.145	5890.03	5374.25
2	8706.111	9970.665	7161.696	9967.934	6024.036	8896.166	5944.051	7448.352	5400.212	5693.55	5828.785	5532.673	5666.525	5203.567
3	8211.227	9399.645	6658.216	8400.0534	5728.088	8473.734	5734.063	6836.972	5179.008	5226.8	5869.145	5304.265	5395.137	4996.035
4	7815.811	8869.149	6304.546	7651.414	5400.212	7885.686	5514.251	6511.552	4798.328	4856.045	5567.057	5106.557	5150.765	4684.123
5	7465.831	8458.997	6163.168	7619.046	5176.552	7862.188	5494.603	4311.92	4515.888	4580.973	5267.425	4854.817	4657.109	4617.811
6	7142.867	8072.177	5969.144	7029.238	4561.488	75374.16	5190.05	6182.284	4271.516	4362.389	4836.57	4502.39	4442.209	4368.527
7	6878.847	7724.653	5671.968	6777.498	4221.332	7015.035	4994.807	5962.472	4398.897	4180.645	4581.137	4179.417	4318.19	4121.6
8	6619.73	7417.653	5862.308	6335.418	4239.588	6678.58	4677.983	5724.24	4296.076	3821.005	4340.447	4133.99	3920.309	3800.127
9	6388.875	7129.073	5276.552	6001.238	3814.7	6389.98	4497.467	5587.932	3887.316	3878.557	4151.337	3878.557	3689.445	3716.45
10	6175.203	6862.597	4962.184	5705.288	3513.84	6136.848	4224.851	5385.312	3807.332	3866.809	4188.177	3866.277	3470.87	3402.091

Table 4.8. Rheological parameters of SB and SF

Parameters	8± 0.5ºC				25± 0.5ºC				40± 0.5ºC				40± 0.5ºC&75%RH			
Time	Flow Index		C.F		Flow Index		C.F		Flow Index		C.F		Flow Index		C.F	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F
Zero hour	0.51	0.48	99.7	99.8	0.50	0.48	99.7	99.8	0.50	0.48	99.7	99.8	0.52	0.48	99.7	99.9
15 days	0.49	0.24	99.8	99.4	0.52	0.47	99.8	98.6	0.43	0.41	99.7	99.8	0.46	0.48	99.9	99.6
30 days	0.58	0.41	99.7	99.3	0.52	0.41	99.2	99.4	0.42	0.41	99.2	98.3	0.42	0.52	98.1	98.3
45 days	0.58	0.49	99.4	99.7	0.50	0.41	98.7	99.4	0.41	0.35	99.1	99.2	0.39	0.54	98.4	99.7
60 days	0.58	0.46	99.6	99.3	0.52	0.35	98.8	99.8	0.42	0.35	98.8	99.2	0.41	0.58	98.7	98.8
75 days	0.63	0.48	99.8	99.2	0.58	0.38	99.2	99.4	0.47	0.38	99.1	98.8	0.53	0.62	99.2	98.6
90 days	0.65	0.49	99.6	99.1	0.58	0.41	99.3	99.7	0.58	0.45	97.1	99.2	0.50	0.63	98.1	99.1

B= Base; F= Formulation; C.F. = Confidence of Fit

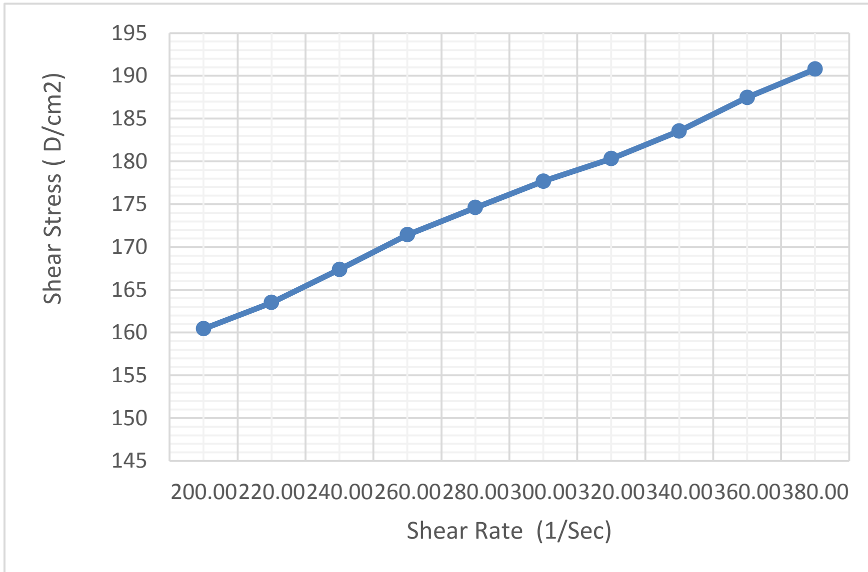


Figure: 4.1. Rheogram of SB at zero hour

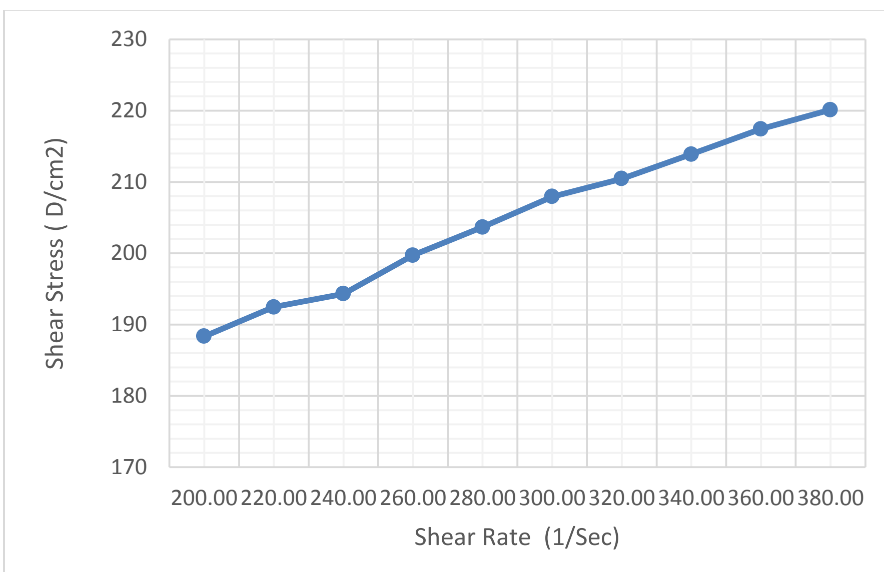


Figure: 4.2. Rheogram of SF at zero hour

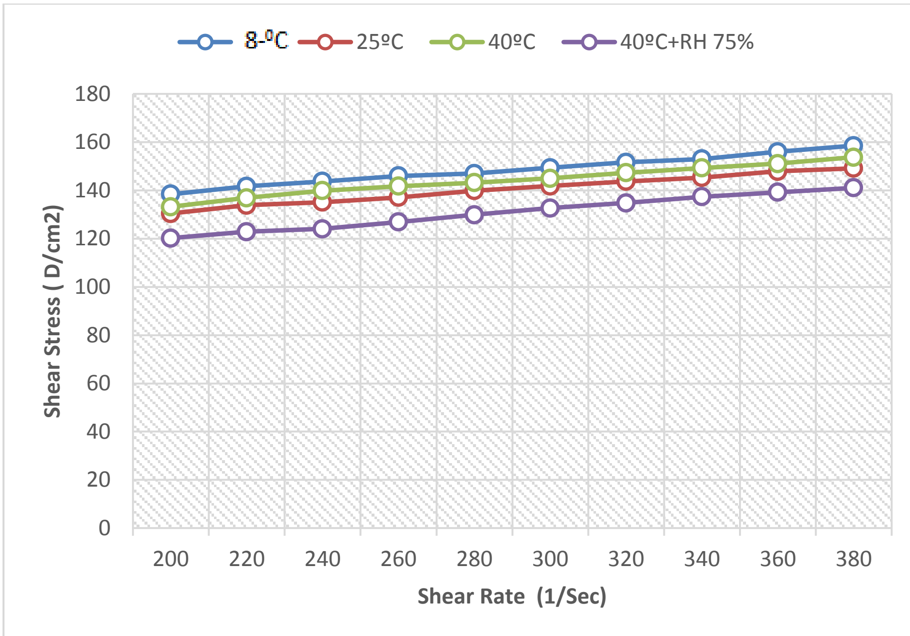


Figure: 4.3.Rheogram of SB at different temperatures after 15 days

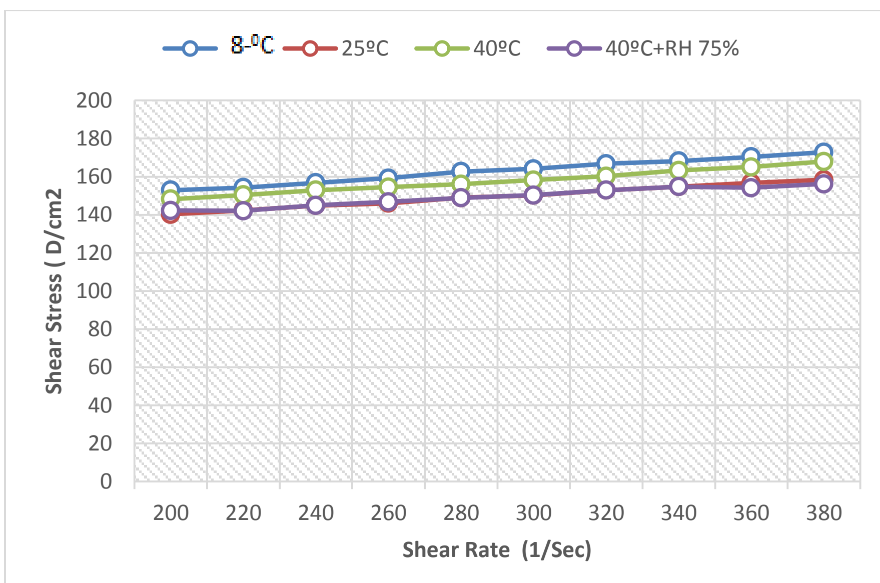


Figure:4.4. Rheogram of SF at different temperatures after 15 days

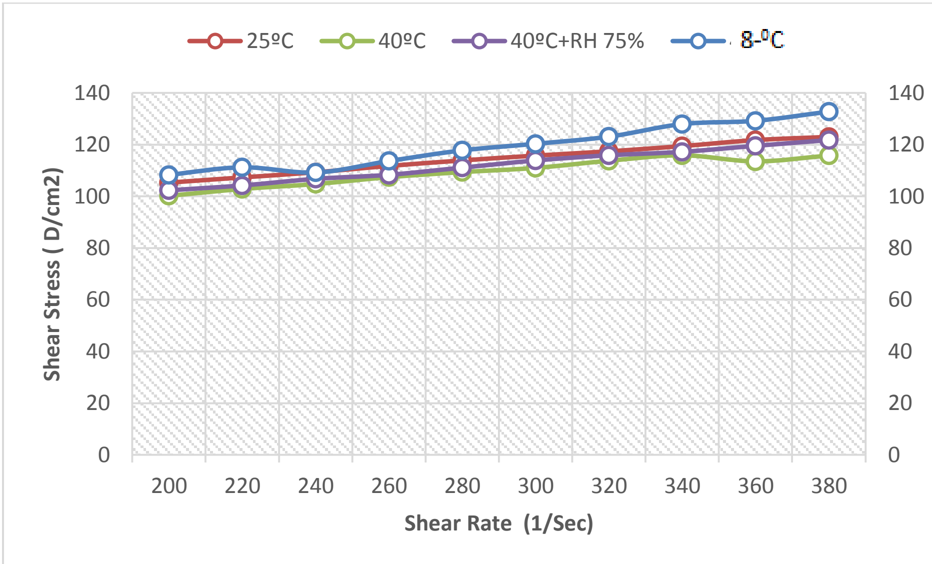


Figure: 4.5.Rheogram of SB at different temperatures after 30 day

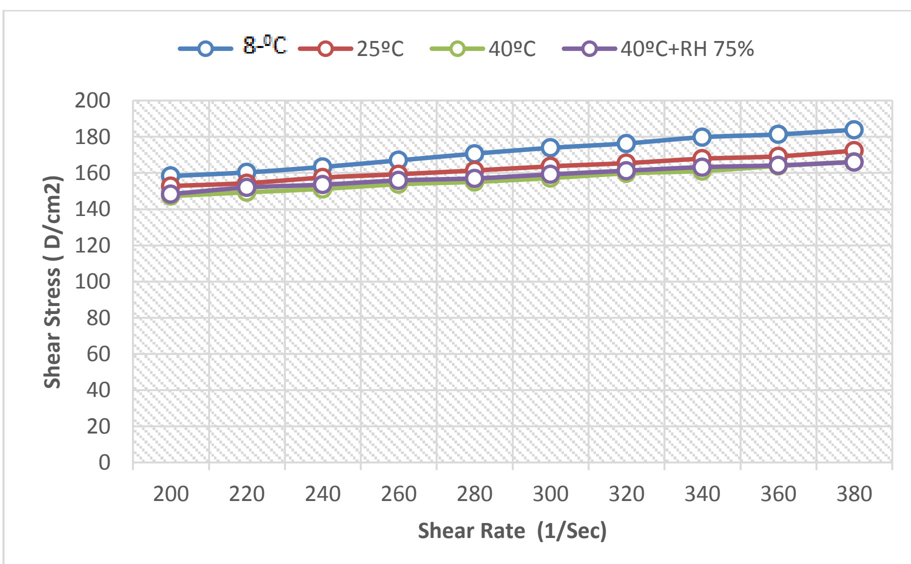


Figure: 4.6. Rheogram of SF at different temperatures after 30 days

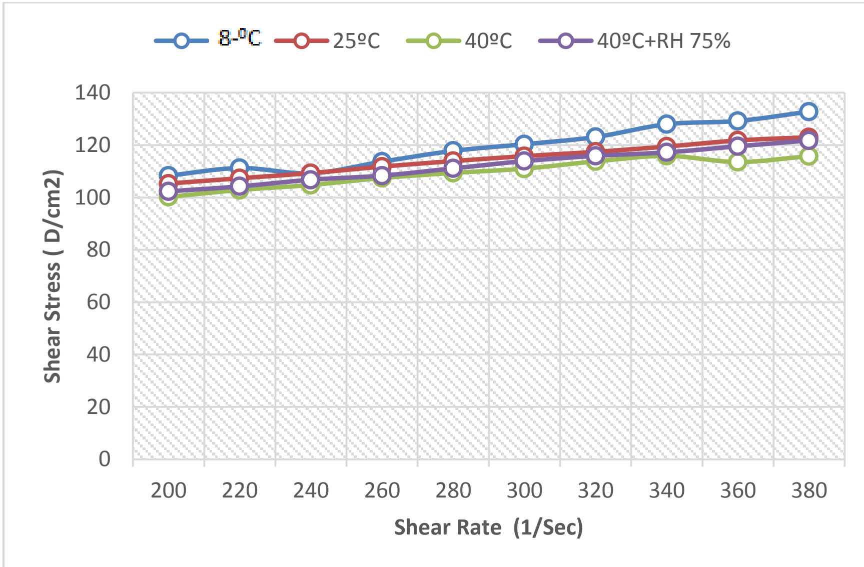


Figure: 4.7. Rheogram of SB at different temperatures after 45 days

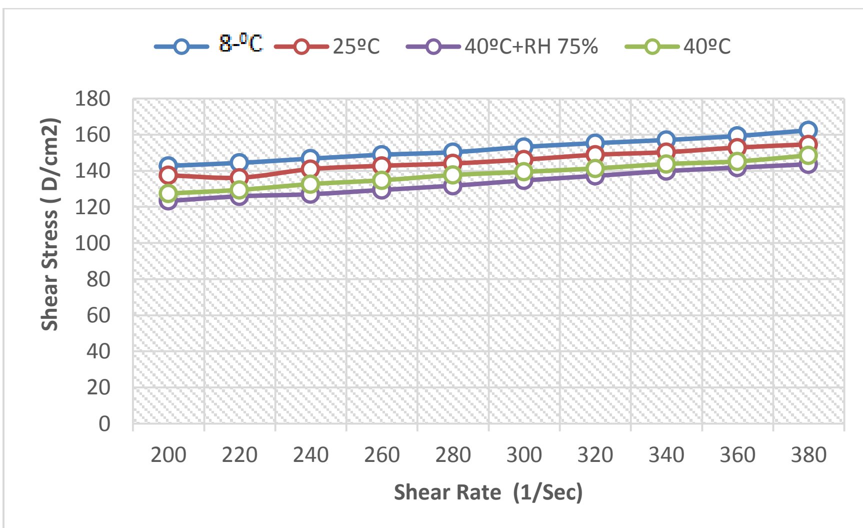


Figure: 4.8. Rheogram of SF at different temperatures after 45 days

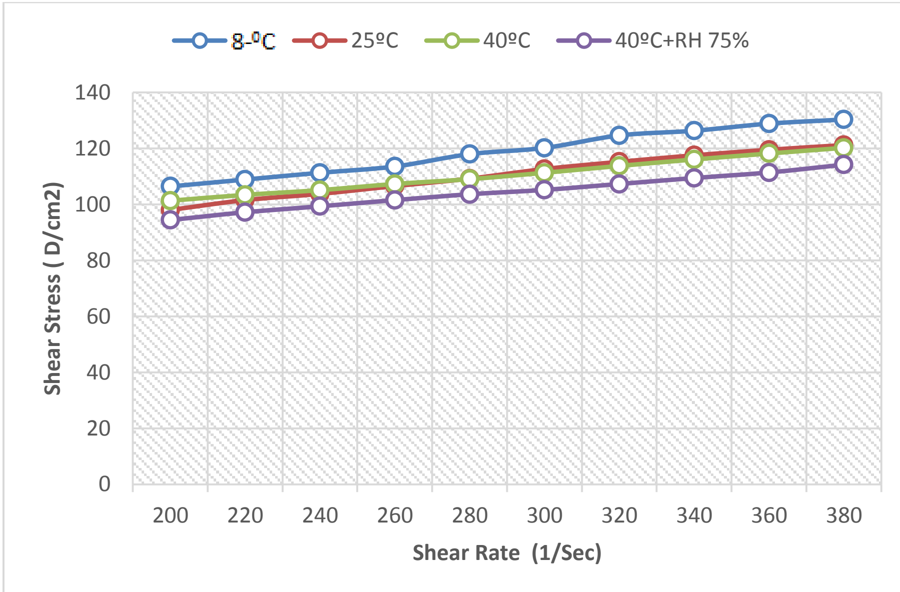


Figure: 4.9.Rheogram of SB at different temperatures after 60 days

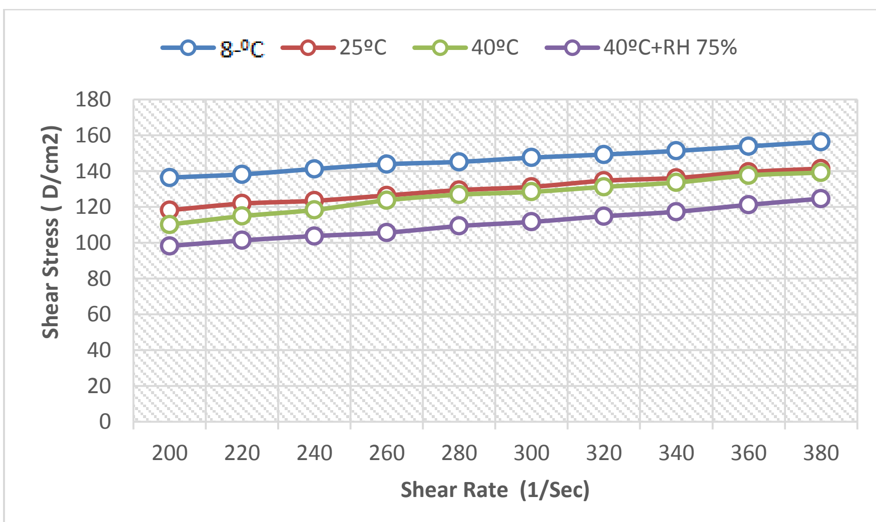


Figure: 4.10. Rheogram of SFat different temperatures after 60 days

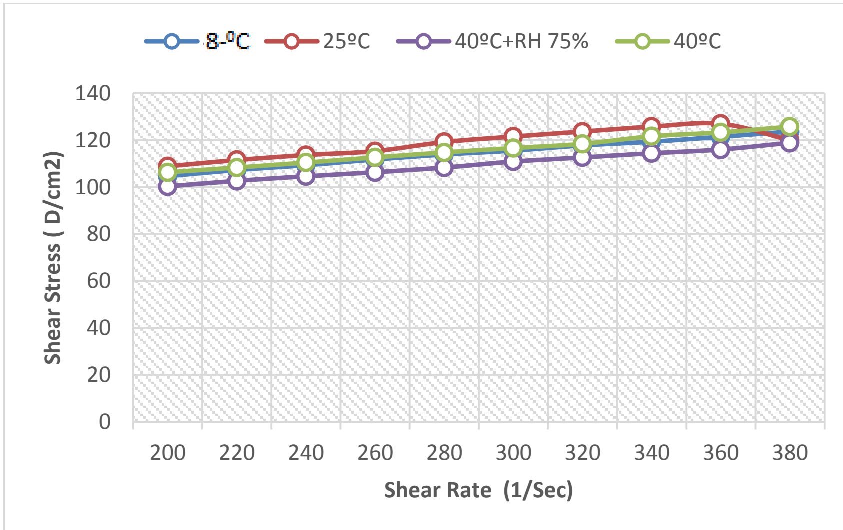


Figure: 4.11. Rheogram of SB at different temperatures after 75days

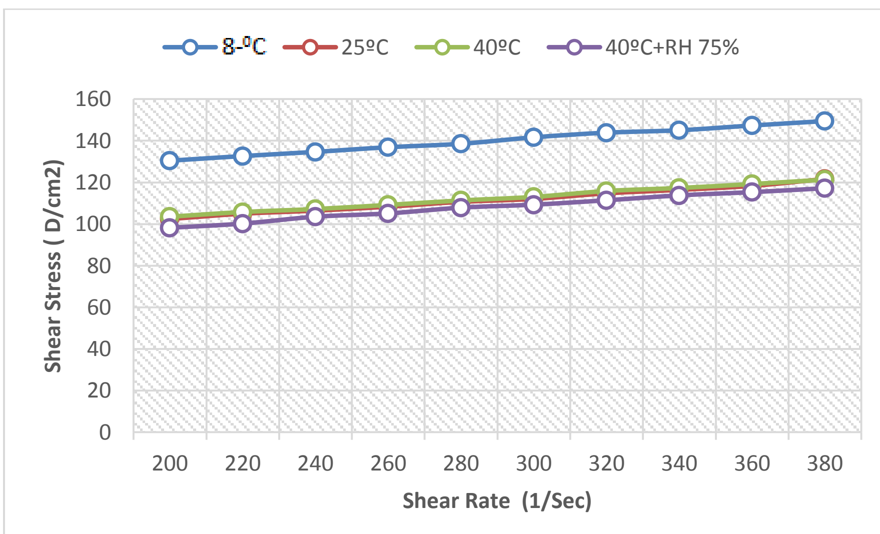


Figure: 4.12.Rheogram of SF at different temperatures after 75days

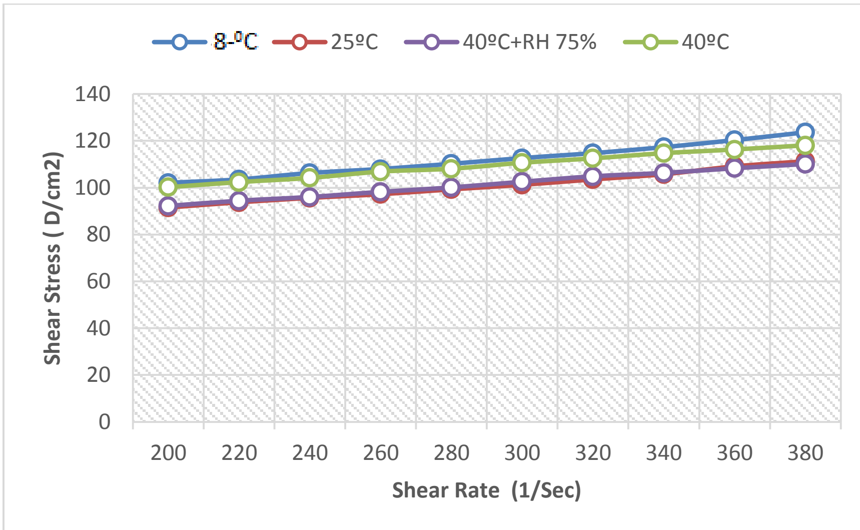


Figure: 4.13. Rheogram of SB at different temperatures after 90days

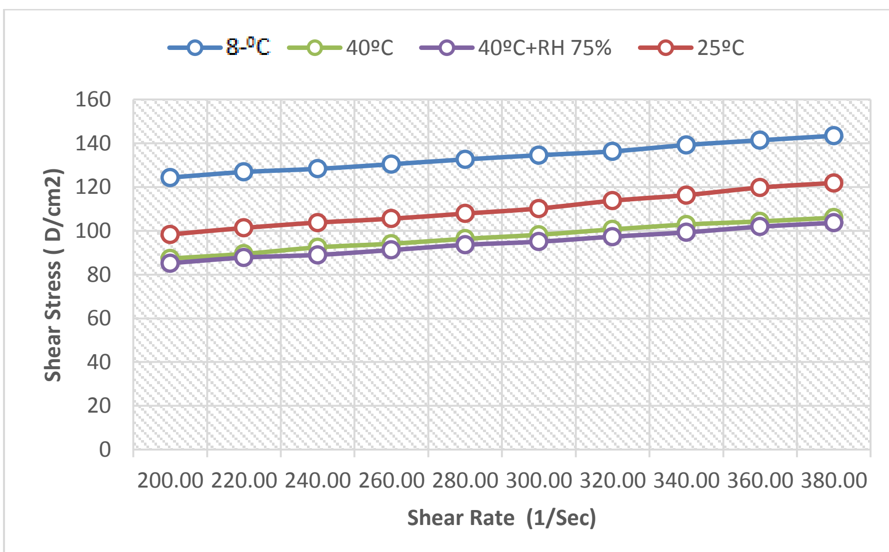


Figure: 4.14. Rheogram of SF at different temperatures after 90days

Table 4.9. Viscosities (cP) of GB and GF kept at 8 ±0.5ºC

Sr.No	Zero hour		15 days		30 days		45 days		60 days		75days		90 days	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	9835.747	10582.4	8300.214	10158.83	7225.01	10721.67	6898.371	9541.14	6474.711	9870.131	5914.744	9741.724	5546.34	9448.927
2	9405.947	9833.41	8186.543	9538.14	6953.631	10075.74	6569.267	9062.22	6232.795	9505.415	5665.45	9265.26	5301.971	9014.215
3	9150.523	9587.81	7969.187	9054.31	6638.035	9550.156	6338.403	9488.223	5971.231	9068.247	5243.028	8830.548	5056.371	8714.583
4	8983.515	9873.815	7468.163	9497.514	6080.523	9071.236	5821.415	9052.283	5513.187	8681.427	4852.524	8405.66	3948.715	8068.122
5	8241.803	9695.755	7070.291	9084.906	5635.987	8627.928	5465.295	8624.93	5273.727	8031.815	4729.723	8022.524	3965.907	7950.767
6	7809.547	9070.703	6867.671	8687.034	5740.367	8173.568	5520.555	8163.906	4998.655	7942.171	4565.171	7790.432	3793.987	7609.383
7	7772.707	8780.895	6646.631	8301.442	5235.65	7729.032	5289.691	7883.227	4776.387	7350.275	4244.663	7422.032	3684.695	7061.695
8	7395.711	8362.147	6380.155	7917.06	5486.171	7264.848	5045.31	7457.110	4552.891	7265.543	3999.063	7216.956	3733.815	6737.503
9	7173.443	7909.015	6080.523	7506.926	5369.510	6735.58	4782.527	7045.731	4238.523	6859.075	3668.293	6921.008	3665.048	6489.447
10	7037.668	7321.336	5712.123	7094.30	5274.955	6261.572	4554.11	6575.407	4046.955	6571.723	3674.872	6890.308	3611.547	6189.815

Table 4.10. Viscosities (cP) of GB and GF kept at 25 ±0.5ºC

Sr.No	Zero hour		15 days		30 days		45 days		60 days		75 days		90 days	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	9734.746	10582.4	8713.355	9666.96	7953.223	9970.132	7936.031	9128.412	6450.151	8268.815	6025.263	7243.446	5640.923	7116.955
2	9305.947	9833.41	8428.45	9200.32	7317.109	9605.416	7602.015	8920.887	5985.967	8015.851	5589.323	7124.323	5274.955	6898.371
3	9050.523	9587.81	8168.123	8928.950	6849.251	9168.248	7118.183	8645.815	5766.155	7728.589	5398.983	6937.667	5152.155	6626.983
4	8883.515	9773.815	7936.031	8345.814	6500.549	8781.428	6898.371	8391.611	5349.863	7463.251	5274.955	6743.643	4852.523	6462.431
5	8141.802	9695.755	7690.431	8177.414	6290.144	8131.816	6571.723	8139.871	4770.247	7202.915	5146.015	6568.047	4729.723	6229.110
6	7709.546	9070.703	7446.05	7918.306	6002.235	8042.172	5578.271	7902.875	4497.631	6976.963	4757.967	6383.835	4511.143	6001.931
7	7672.706	8780.895	7076.431	7140.982	5649.495	7450.276	5521.783	7657.275	4167.313	6742.415	4497.631	6224.263	4292.555	5762.471
8	7295.711	8362.147	6823.463	6949.414	5453.015	7165.543	5262.675	7431.323	4043.271	6534.883	4292.555	6055.963	3992.923	5534.063
9	7173.442	7909.015	6511.551	6906.434	5287.235	6759.075	5076.01	7205.371	3801.355	6326.123	4113.267	5898.771	3843.107	5249.167
10	6937.667	7221.335	6259.811	6632.590	5114.087	6471.722	5023.215	6988.015	3678.555	6122.275	3857.843	5748.963	3665.047	4729.723

Table 4.11. Viscosities (cP) of GB and GF kept at 40 ±0.5ºC

Sr.No	Zero hour		15 days		30 days		45 days		60 days		75days		90 days	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	9934.747	9582.412	8427.231	9626.987	7676.923	9073.271	8290.923	9870.131	6399.108	8676.517	6216.831	6817.323	5952.811	6792.067
2	9504.947	9733.413	8180.403	9181.223	7431.323	9564.367	7922.523	9505.415	6182.98	8316.713	6134.555	6624.527	5712.123	6571.723
3	9250.523	9387.803	7922.523	8746.513	7188.179	9186.135	7708.851	9068.247	5890.716	8030.589	5875.447	6816.095	5454.243	6381.383
4	9083.515	9773.815	7630.954	8332.675	6830.831	8854.575	7419.043	8681.427	5083.92	7753.058	5589.323	6556.987	5220.923	6091.575
5	8341.803	9795.755	7358.345	7924.971	6558.215	8548.803	7173.443	8031.815	4914.456	7505.004	5220.923	6324.895	4810.771	5847.204
6	7909.547	8970.702	7285.724	7519.745	6326.123	8254.083	6940.123	7942.171	4829.724	7279.052	4862.347	6094.031	4556.575	5594.236
7	7872.707	8680.895	7079.424	7100.991	5957.723	7986.386	6572.951	7350.275	4593.948	7072.748	4603.238	5828.783	4212.735	5383.029
8	7495.71	8362.147	6796.97	6650.315	5685.107	7723.587	6202.095	7265.543	4316.42	6872.584	4415.354	5583.183	3988.011	5112.851
9	7273.443	7909.015	6566.117	6501.727	5465.295	7481.671	5957.723	6859.0751	4040.815	6685.928	4169.755	5346.178	3733.815	4839.015
10	7037.667	7221.335	6316.831	6027.71	5228.291	7240.983	5520.555	6671.724	4113.8	6618.93	3980.643	5111.631	3676.119	4579.907

Table 4.12. Viscosities (cP) of GB and GF kept at 40±0.5ºC+ 75%RH

Sr.No	Zero hour		15 days		30 days		45 days		60 days		75days		90 days	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	11299.25	11570.63	7608.526	9258.425	6728.745	3200.177	6206.845	7942.009	5892.477	6192.109	6254.737	5869.145	5890.0214	5374.26
2	9806.113	9970.665	7361.698	8867.934	6224.037	8896.165	5944.053	7448.353	5600.213	5693.556	6028.784	5532.673	5666.528	5203.568
3	9311.229	9399.645	6758.218	8400.053	5928.089	8473.733	5734.065	7036.973	5179.009	5226.912	5869.145	5304.265	5295.137	4996.036
4	8915.813	8869.149	6504.555	8051.378	5600.213	7985.685	5514.253	6711.553	4798.329	4856.045	5567.056	5106.557	5250.766	4684.124
5	8565.833	8458.997	6163.168	7619.045	5176.553	7862.189	5494.605	6411.932	4515.889	4580.973	5267.425	4854.817	4757.109	4617.812
6	8342.869	8072.175	5969.146	7329.237	4761.489	7474.14	5190.071	6182.285	4271.517	4362.389	4936.569	4502.396	4542.209	4368.528
7	7978.849	7724.653	5771.969	6877.497	4421.333	7215.033	4994.809	5962.473	4198.087	4180.645	4781.137	4179.417	4418.198	4121.7
8	7719.75	7417.651	5962.309	6635.417	4239.589	6878.57	4677.985	5724.241	4296.077	3921.005	4740.449	4133.995	3820.309	4000.128
9	7388.877	6929.073	5276.554	6101.237	3814.754	6589.984	4497.469	5587.933	4087.317	3878.557	4351.337	3878.557	3789.445	3716.46
10	7275.205	6862.597	4962.186	5805.289	3513.863	6136.849	4324.853	5385.313	3807.333	3766.809	4388.177	3866.277	3570.878	3402.092

Table 4.13. Rheological parameters of GB and GF

Parameters	8± 0.5ºC				25± 0.5ºC				40± 0.5ºC				40± 0.5ºC+75%RH			
Time	Flow Index		C.F		Flow Index		C.F		Flow Index		C.F		Flow Index		C.F	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F
Zero hour	0.45	0.37	99.8	99.4	0.45	0.39	99.8	99.4	0.45	0.36	99.8	99.4	0.47	0.37	99.8	99.4
15 days	0.47	0.37	99.8	99.1	0.46	0.43	99.8	99.1	0.48	0.47	99.7	99.6	0.47	0.41	99.7	98.8
30 days	0.47	0.36	99.7	99.1	0.46	0.48	99.2	99.1	0.48	0.51	99.3	98.8	0.48	0.46	98.1	99.0
45 days	0.48	0.40	99.6	99.1	0.47	0.52	99.7	99.4	0.50	0.53	99.1	99.2	0.50	0.47	98.4	99.1
60 days	0.50	0.40	99.7	99.0	0.51	0.63	99.8	99.1	0.51	0.53	99.2	99.6	0.51	0.47	98.5	98.6
75 days	0.50	0.44	99.8	99.4	0.52	0.63	99.4	99.1	0.54	0.53	99.7	99.0	0.54	0.50	99.0	99.0
90 days	0.52	0.46	99.6	99.4	0.53	0.66	99.7	99.8	0.58	0.56	98.1	99.4	0.57	0.52	98.1	99.4

B= Base; F= Formulation; C.F. = Confidence of F

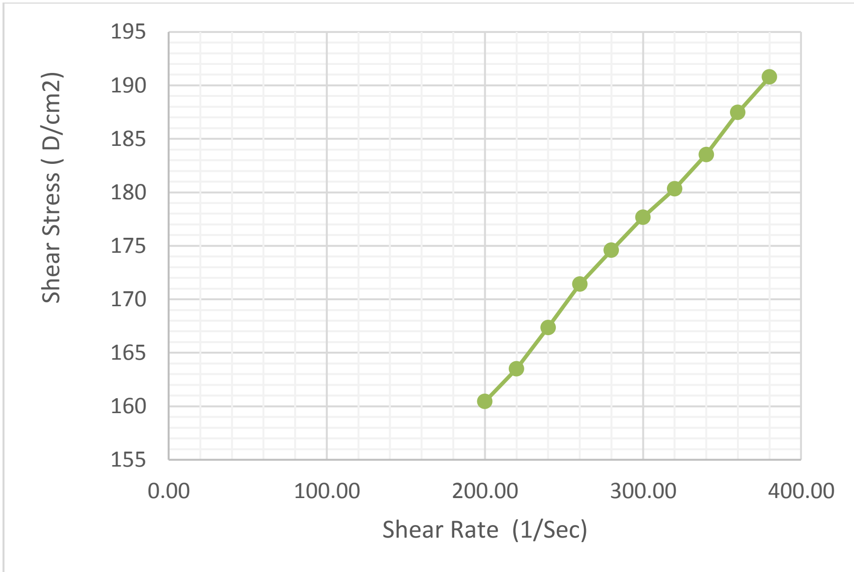


Figure: 4.15. Rheogram of GB at zero hour

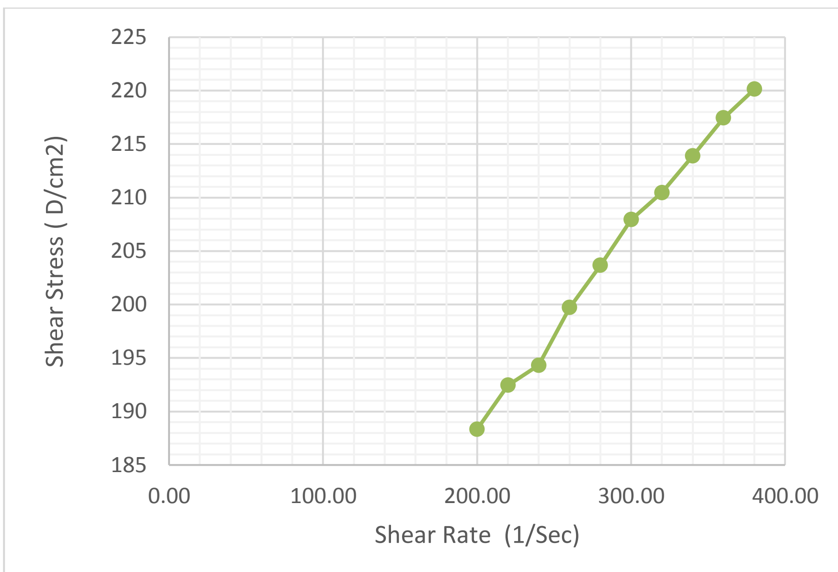


Figure: 4.16. Rheogram of GF at zero hour

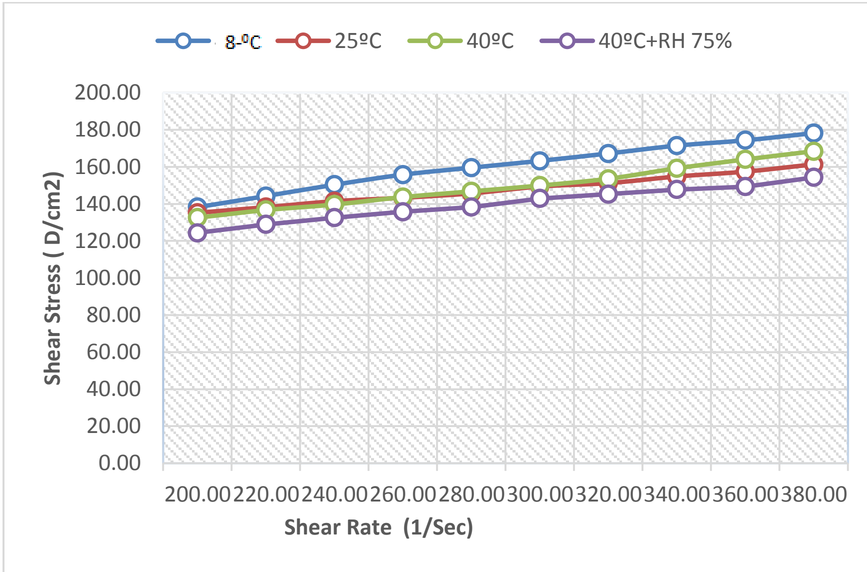


Figure: 4.17. Rheogram of GB at different temperatures after 15 days

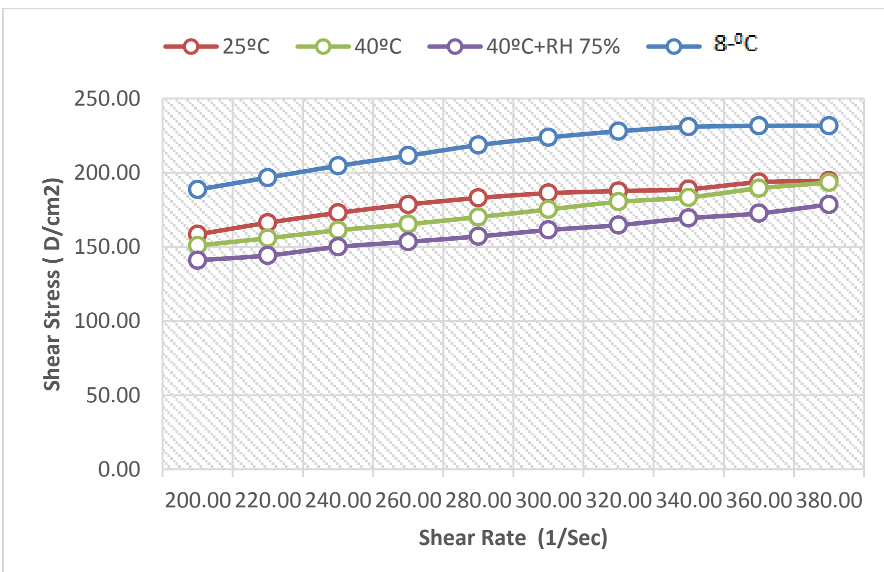


Figure: 4.18. Rheogram of GF at different temperatures after 15 days

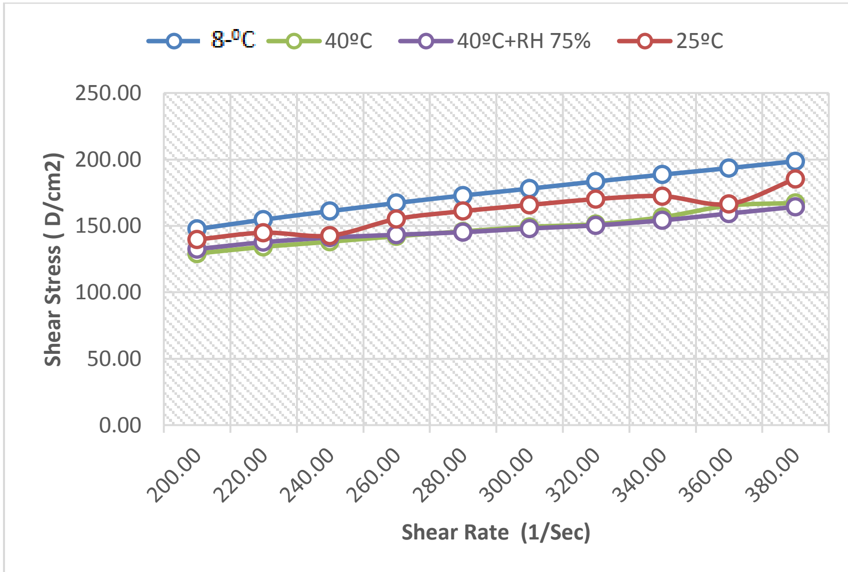


Figure: 4.19. Rheogram of GB at different temperatures after 30 days

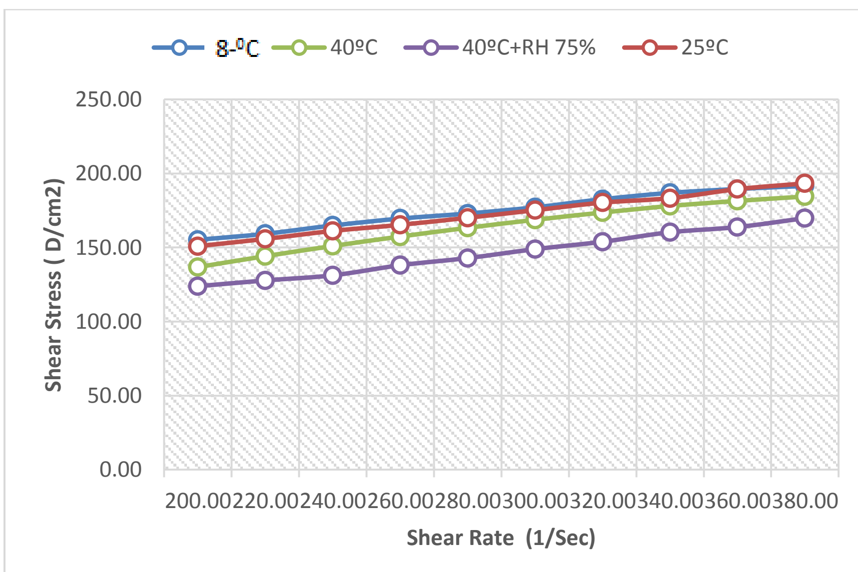


Figure: 4.20.Rheogram of GF at different temperatures after 30 days

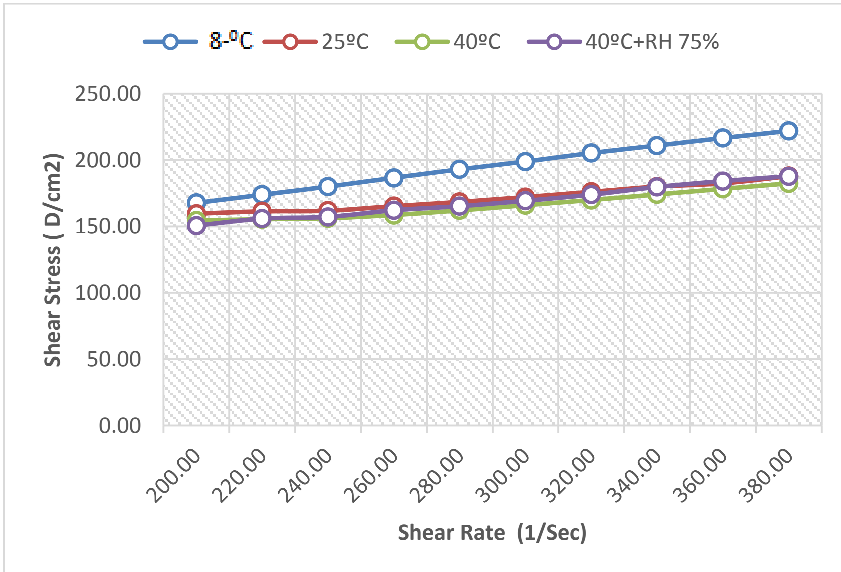


Figure: 4.21. Rheogram of GB at different temperatures after 45 days

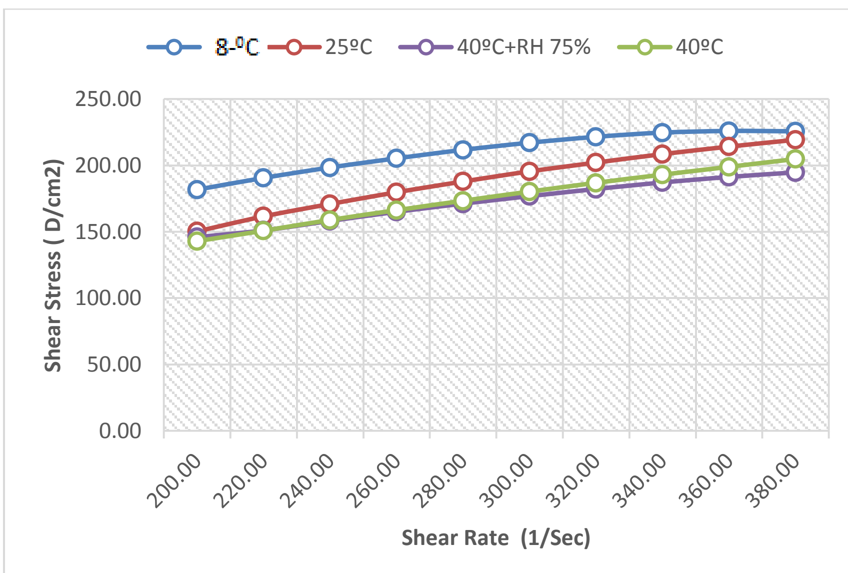


Figure: 4.22. Rheogram of GF at different temperatures after 45 days

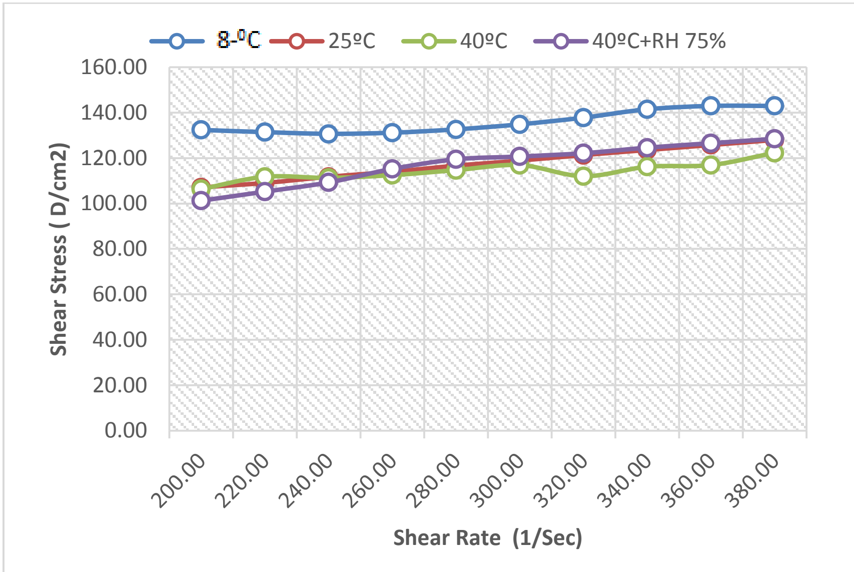


Figure: 4.23. Rheogram of GB at different temperatures after 60 days

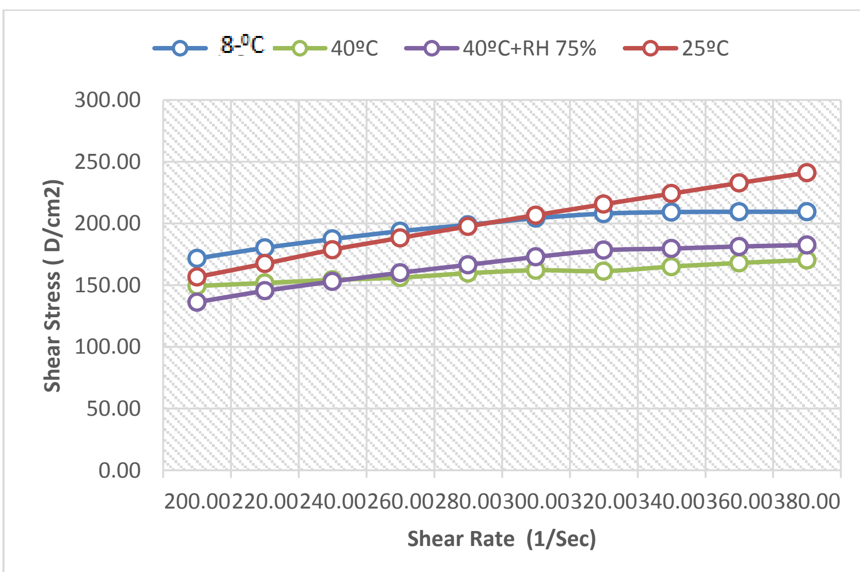


Figure: 4.24. Rheogram of GF at different temperatures after 60 days

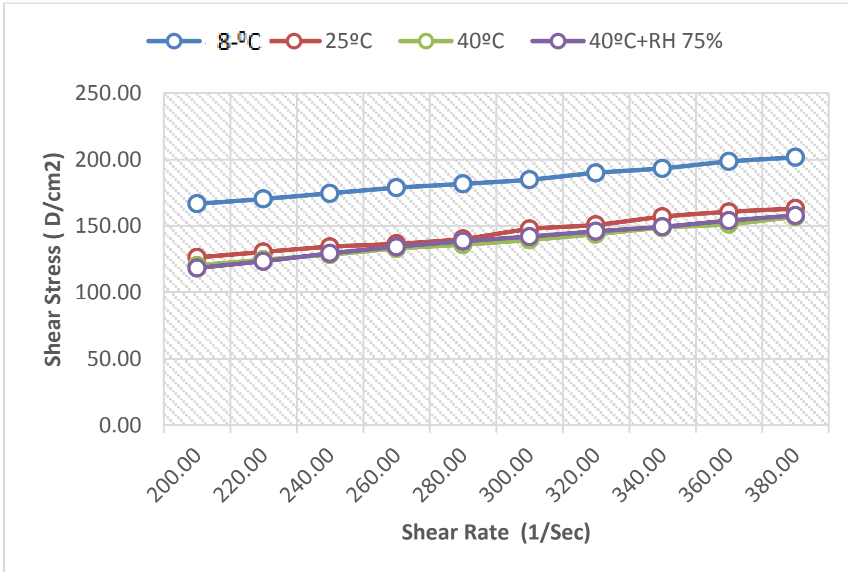


Figure: 4.25. Rheogram of GB at different temperatures after 75 days

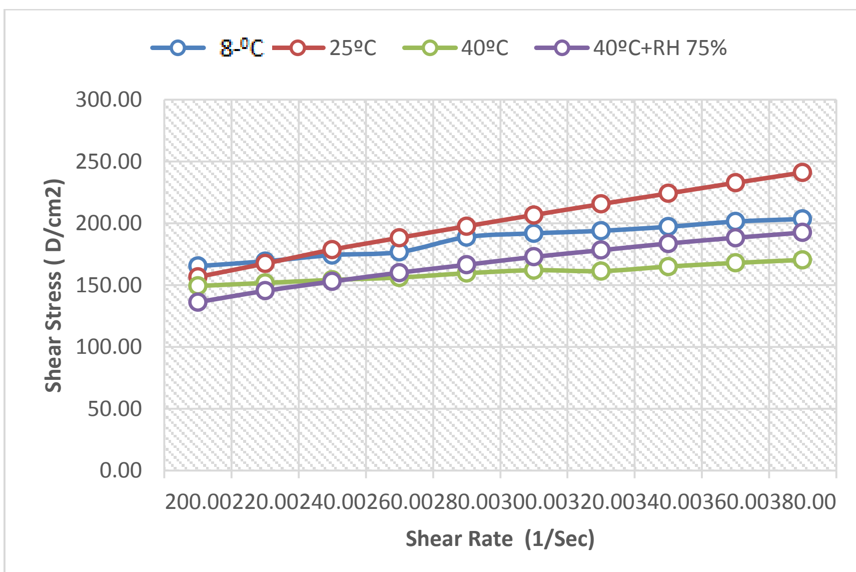


Figure: 4.26.Rheogram of GF at different temperatures after 75 days

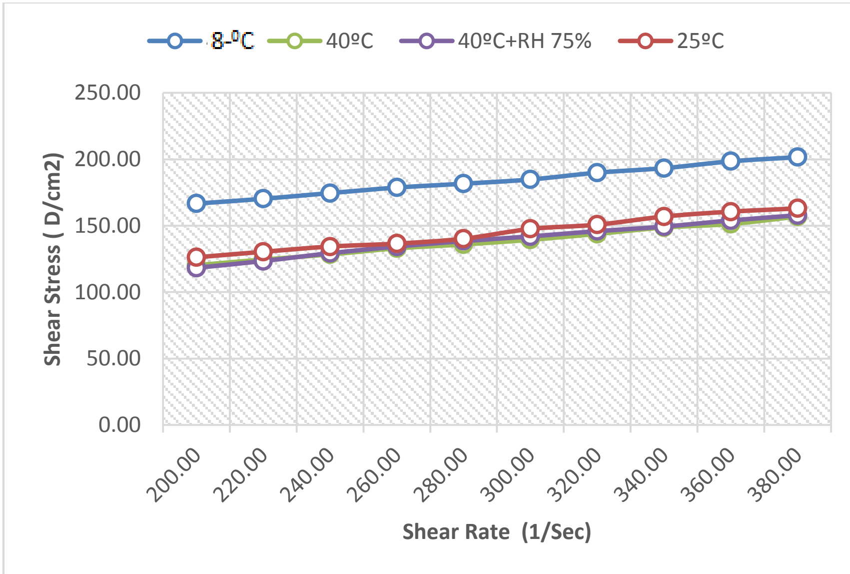


Figure: 4.27. Rheogram of GB at different temperatures after 90 days

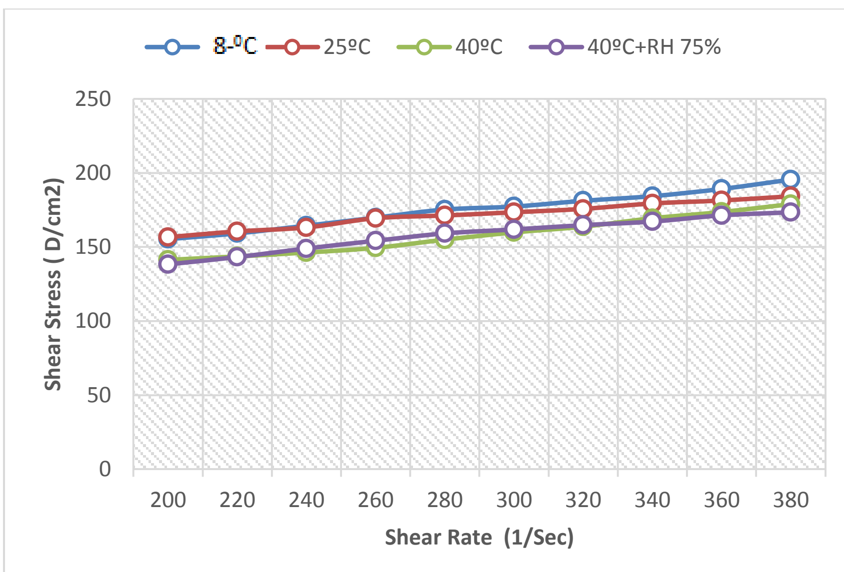


Figure: 4.28.Rheogram of GF at different temperatures after 90 days

Table 4.14. Viscosities (cP) of TB and TF kept at 8±0.5ºC º

Sr.No	Zero hour		15 days		30 days		45 days		60 days		75days		90 days	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	9393.667	11299.13	9337.017	11124.86	8490.925	9898.037	7644.832	8190.065	7153.633	7976.228	6457.357	7032.07	6039.837	7146.265
2	9014.215	11005.74	9043.525	9976.804	8170.417	9488.063	7253.156	7935.869	6772.953	7723.267	6037.388	6701.729	5729.153	6949.785
3	8632.307	9841.724	8654.249	9610.86	7771.317	9228.953	6894.524	7659.569	5833.697	7482.574	5678.805	6360.345	5350.929	6704.185
4	8185.315	9669.804	8269.885	9392.276	7291.169	8934.233	6469.636	7385.725	5829.849	7286.094	5369.349	5940.369	4935.865	6445.077
5	7799.723	9393.504	7957.973	9173.692	6928.909	8505.67	6014.048	7141.353	5424.609	6972.954	4998.493	5585.477	4547.817	6063.169
6	7426.411	9171.236	7644.833	8901.076	6529.809	8172.873	5682.488	6830.669	4851.297	6677.006	4560.097	5217.077	3993.989	5608.276
7	7062.923	8902.304	7385.725	8543.728	5837.391	7773.773	5377.944	6597.349	4667.097	6144.054	4377.125	4763.945	3884.697	5325.836
8	6658.911	8509.344	6911.717	8314.092	5710.731	7384.497	5053.752	6158.953	4291.329	5785.478	4141.349	4309.585	3933.817	4819.99
9	6326.123	8277.252	6512.617	8014.46	5458.991	7141.353	4736.928	5799.149	4108.193	5403.569	3834.392	3941.185	3865.049	4600.089
10	5821.415	8030.424	6416.833	7782.368	4820.595	6756.989	4336.634	5598.985	3776.797	4959.728	3592.433	3714.005	3711.549	4220.637

Table 4.15. Viscosities (cP) of TB and TF kept at 25±0.5C °

Sr.No	Zero hour		15 days		30 days		45 days		60 days		75days		90 days	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	9515.315	11299.25	9090.189	9971.893	8295.673	9319.292	7730.098	8005.865	7002.589	7291.169	6267.017	6675.408	6138.772	6799.969
2	9214.217	11005.75	8736.525	9720.153	7875.697	9033.168	7365.38	7872.546	6547.696	7067.673	5881.589	6417.528	5788.097	6422.9734
3	8832.309	9841.725	8509.345	9392.277	7589.573	8747.044	6809.279	7550.809	6375.776	6791.373	5788.097	6211.224	5589.671	6209.303
4	8385.317	9669.805	8239.185	9021.432	7147.493	8577.58	6696.817	7284.334	5954.572	6545.773	5623.545	5966.852	5255.145	5980.893
5	7999.725	9393.505	8059.897	8754.945	6826.983	8274.264	6491.046	7014.174	5545.648	6333.329	5181.465	5717.568	4792.189	5694.769
6	7626.413	9171.237	7752.897	8387.773	6404.553	8102.344	6136.154	6780.854	5258.296	6042.293	4878.149	5510.036	4568.693	5462.677
7	7262.925	8902.305	7372.217	8067.265	5967.385	7808.852	5846.346	6578.234	4840.776	5866.689	4437.296	5239.876	4309.585	5202.35
8	6858.913	8509.345	7203.99	7649.745	5788.097	7538.692	5403.038	6312.986	4479.744	5585.477	4110.649	5021.292	3942.413	4848.677
9	6426.124	8177.252	6782.777	7260.469	5614.949	7272.216	4909.391	6029.318	4274.668	5328.825	3812.245	4738.852	3781.547	4466.769
10	5921.416	7930.424	6586.297	6974.345	5203.569	7143.809	4649.046	5794.779	3890.304	5121.293	3696.813	4492.024	3467.177	3975.569

Table 4.16. Viscosities (cP) of TB and TF kept at 40±0.5°C

Sr.No	Zero hour		15 days		30 days		45 days		60 days		75days		90 days	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	9393.667	11299.249	8678.973	9738.573	7798.495	8946.677	8109.016	7604.823	7384.497	6835.743	5881.587	6704.184	5856.865	6418.0645
2	90114.215	11005.749	8362.149	9292.277	7490.267	8687.569	7945.692	7550.277	6876.269	6490.675	5665.468	6146.673	5489.693	6224.037
3	8632.307	9841.723	8056.377	9037.385	7070.291	8293.39	7519.576	7126.617	6653.835	5976.143	5256.535	5926.89	5105.329	5940.369
4	8285.315	9669.803	7737.097	8641.969	6710.487	8087.076	6814.868	6813.477	6028.949	5642.127	5175.325	5608.898	4921.668	5705.83
5	7799.723	9393.503	7442.377	8396.369	6332.263	7708.832	6436.644	6420.517	5638.443	5249.167	4991.125	5230.585	4546.589	5349.168
6	7426.411	9171.235	7135.377	8068.493	5821.415	7459.598	5854.572	6004.693	5267.589	4994.973	4615.355	4848.677	4350.109	5228.129
7	7062.923	8902.303	6816.097	7686.585	5588.095	7091.169	5791.78	5831.077	4718.671	4686.743	4375.895	4480.277	3898.369	4875.693
8	6657.911	8509.343	6456.2923	7386.953	5084.615	6832.067	4993.744	5449.916	4481.667	4254.487	3800.127	4098.369	3896.977	4589.569
9	6226.123	8277.251	6177.373	7219.945	4859.891	6613.477	4739.547	5194.973	4295.011	4007.668	3511.547	3760.669	392.433	4300.989
10	5721.415	8030.423	5635.989	6813.477	4593.415	6367.877	4495.177	4712.369	3889.771	3802.583	3253.667	3470.856	3332.097	3954.693

Table 4.17. Viscosities (cP) of TB and TF kept at 40±0.5ºC º+ 75%RH

Sr.No	Zero hour		15 days		30 days		45 days		60 days		75days		90 days	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	9393.667	11299.23	8795.469	9604.026	8164.972	8654.944	7823.588	8042.172	7052.404	6814.172	6605.945	6281.753	6188.425	6199.477
2	9014.215	11005.75	8292.153	9332.638	7649.212	8410.572	7705.005	7872.013	6666.812	6469.637	6089.652	5940.369	5963.776	5817.569
3	8632.307	9641.723	8040.413	9123.878	7317.652	8164.972	7405.373	7659.569	6335.252	5926.87	5853.876	5585.477	5708.277	5473.729
4	8385.315	9469.803	7789.735	8854.944	6968.9	7970.948	7194.157	7168.369	5826.86	5585.477	5581.26	5193.745	5473.729	5242.865
5	7799.723	9193.503	7229.931	8605.68	6626.288	7686.052	6930.137	6960.837	5585.477	5190.07	5363.904	4821.67	5078.3129	4821.67
6	7426.413	9171.235	6986.787	8277.786	6110.528	7428.172	6655.065	6677.169	5082.692	4739.385	5094.972	4343.969	4858.589	4480.277
7	7062.923	8902.303	6937.505	7983.069	5840.368	7047.492	6427.885	6312.453	4704.468	4357.477	4857.968	4116.093	4589.569	4234.677
8	6658.913	8309.343	6725.07	7750.974	5584.944	6822.768	5961.245	5930.545	4380.276	3989.077	4598.86	3585.065	4307.129	3975.569
9	6326.123	8177.252	6322.972	7334.149	5476.185	6614.008	5703.365	5854.409	4051.867	3497.877	4366.768	3238.769	4122.929	3754.529
10	5821.415	7930.424	6079.828	7051.709	5161.816	6209.997	5473.729	5328.292	3862.065	2993.169	4121.168	2783.876	3800.66	3348.079

Table 4.18. Rheological parameters of TB and TF

Parameters	8± 0.5ºC				25± 0.5ºC				40± 0.5ºC				40± 0.5ºC+75%RH			
Time	Flow Index		C.F		Flow Index		C.F		Flow Index		C.F		Flow Index		C.F	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F
Zero hour	0.22	0.14	99.7	99.6	0.22	0.13	99.8	99.8	0.22	0.13	99.8	99.8	0.22	0.20	99.8	99.9
15 days	0.41	0.20	99.4	99.6	0.33	0.20	99.9	99.9	0.31	0.20	99.9	99.4	0.20	0.20	99.8	99.9
30 days	0.54	0.31	98.7	99.7	0.31	0.23	98.8	99.8	0.37	0.28	99.8	99.4	0.28	0.28	99.9	99.9
45 days	0.45	0.33	99.7	98.2	0.38	0.21	98.8	98.9	0.40	0.28	98.9	99.8	0.30	0.32	99.8	99.8
60 days	0.47	0.38	99.5	99.7	0.40	0.28	96.8	99.8	0.37	0.30	99.8	99.9	0.33	0.42	97.5	99.8
75 days	0.45	0.40	99.7	99.7	0.42	0.42	99.9	99.6	0.46	0.34	97.7	99.6	0.37	0.42	98.8	99.5
90 days	0.48	0.46	99.7	98.6	0.42	0.43	99.8	98.8	0.43	0.38	98.8	98.8	0.40	0.40	97.2	98.5

B= Base; F= Formulation; C.F. = Confidence of Fi

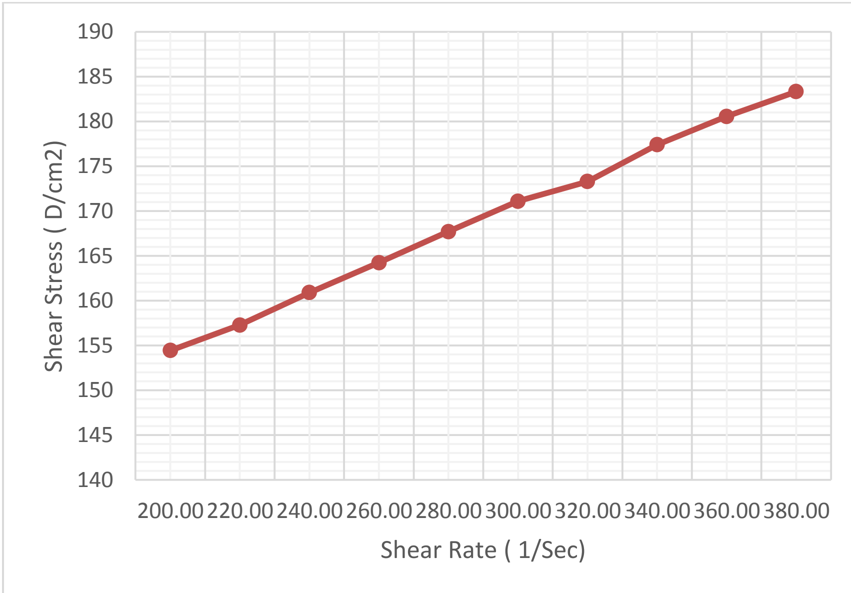


Figure: 4.29. Rheogram of TB at zero hour

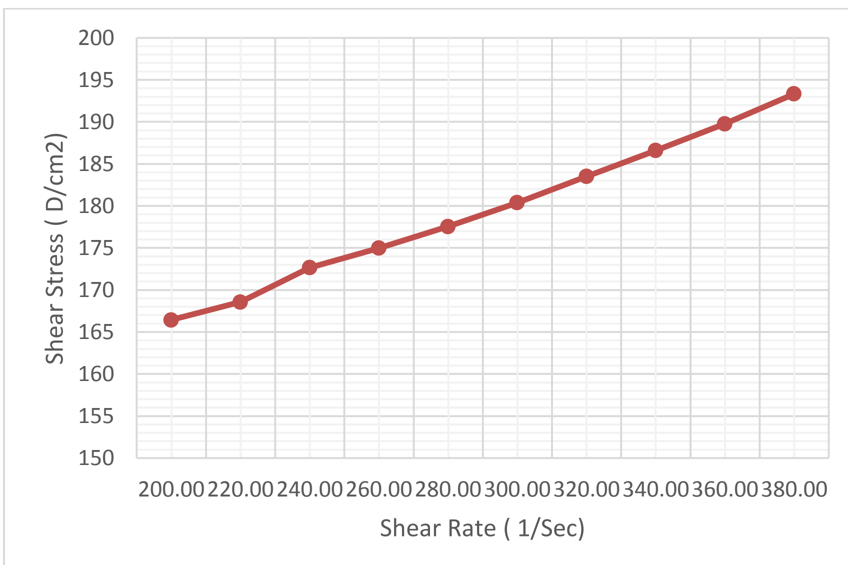


Figure: 4.30. Rheogram of TF at zero hour

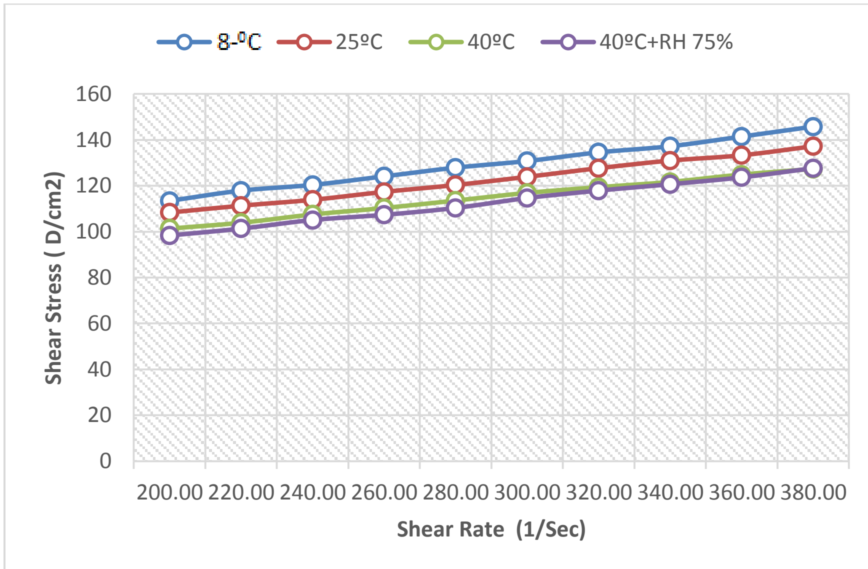


Figure: 4.31. Rheogram of TB at different temperatures after 15 days

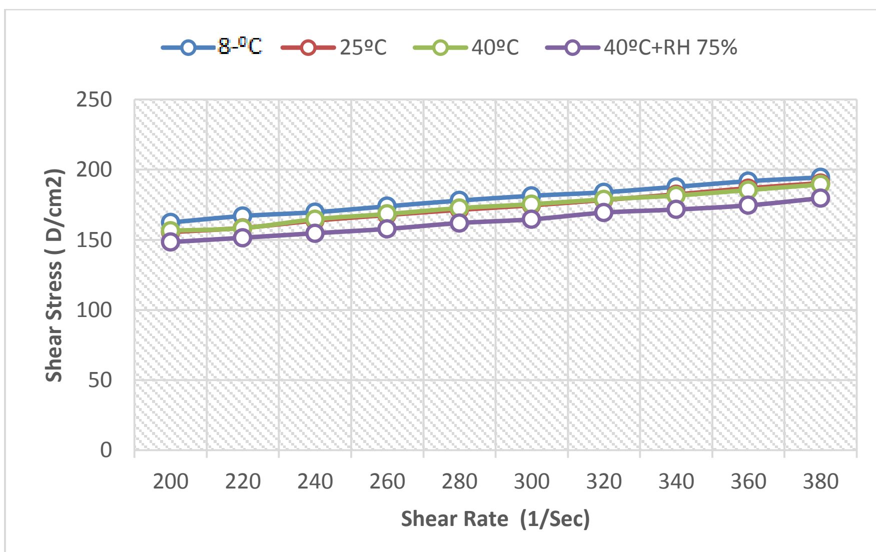


Figure: 4.32. Rheogram of TF at different temperatures after 15 days

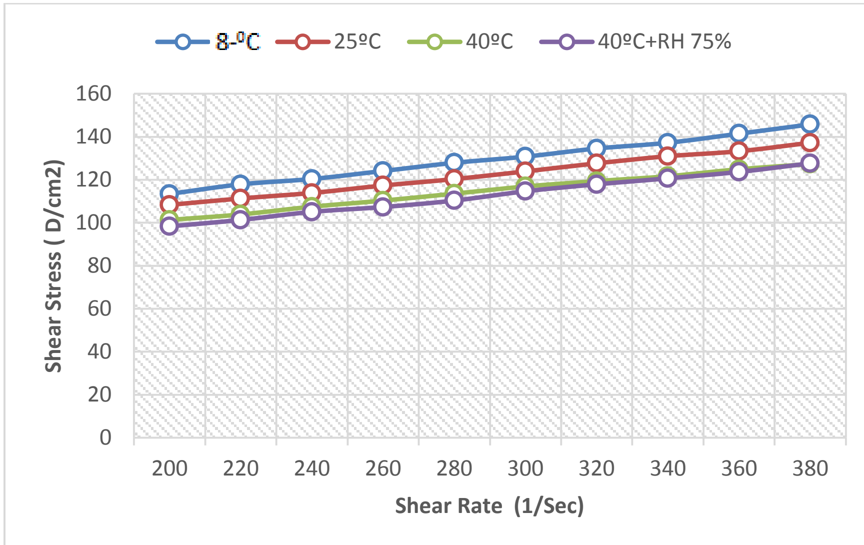


Figure: 4.33. Rheogram of TB at different temperatures after 30 days

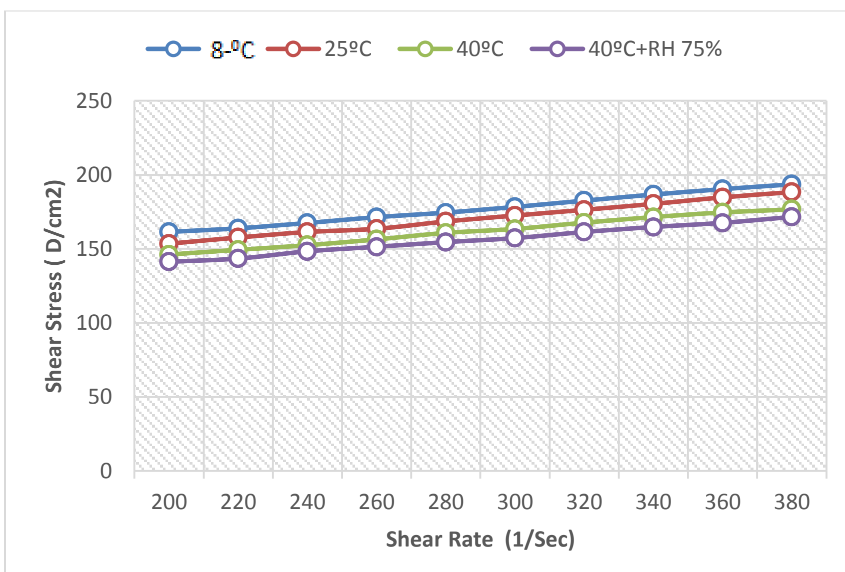


Figure: 4.34. Rheogram of TF at different temperatures after 30 days

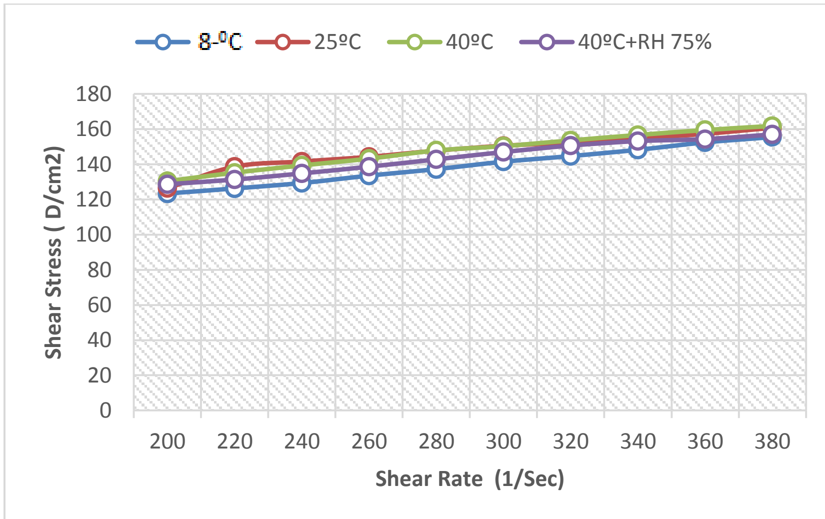


Figure: 4.35. Rheogram of TB at different temperatures after 45 days

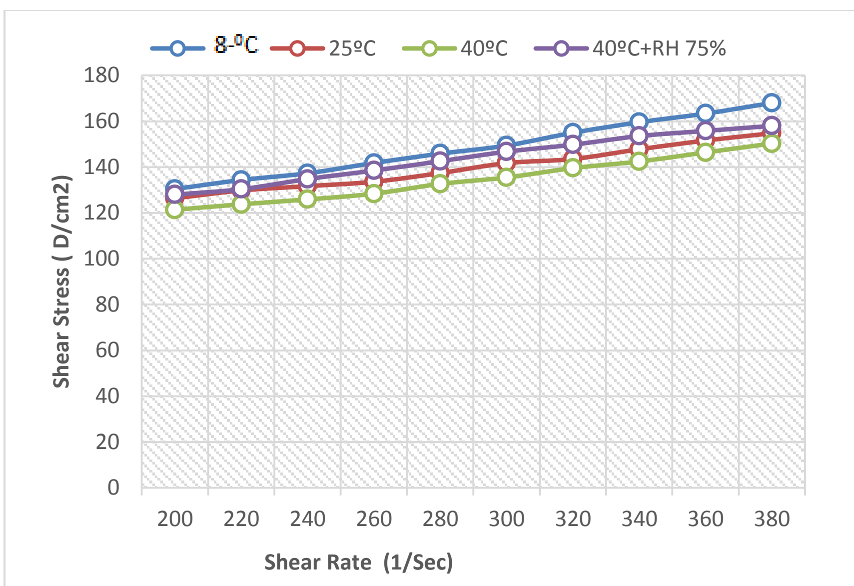


Figure: 4.36. Rheogram of TF at different temperatures after 45 days

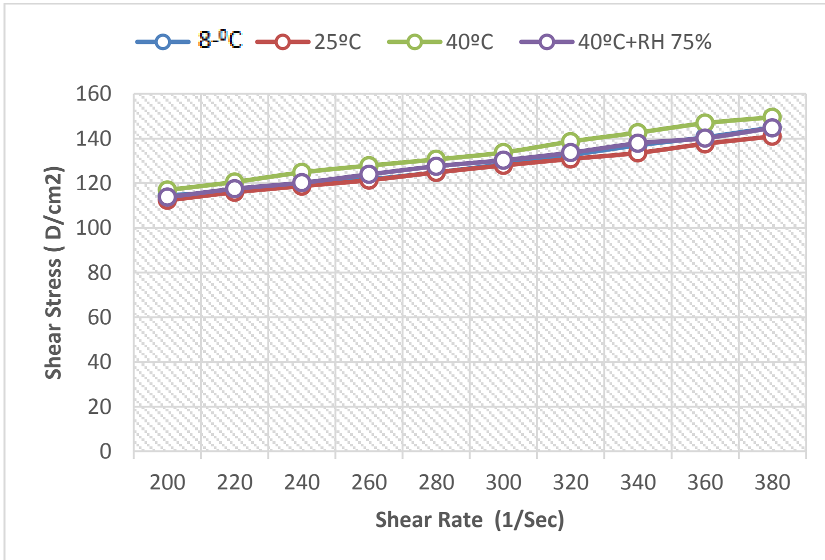


Figure: 4.37. Rheogram of TB at different temperatures after 60 days

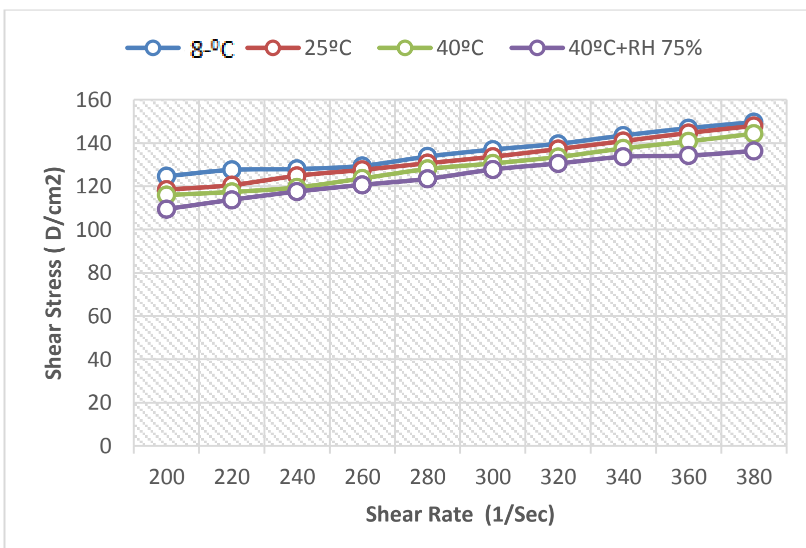


Figure: 4.38. Rheogram of TF at different temperatures after 60 days

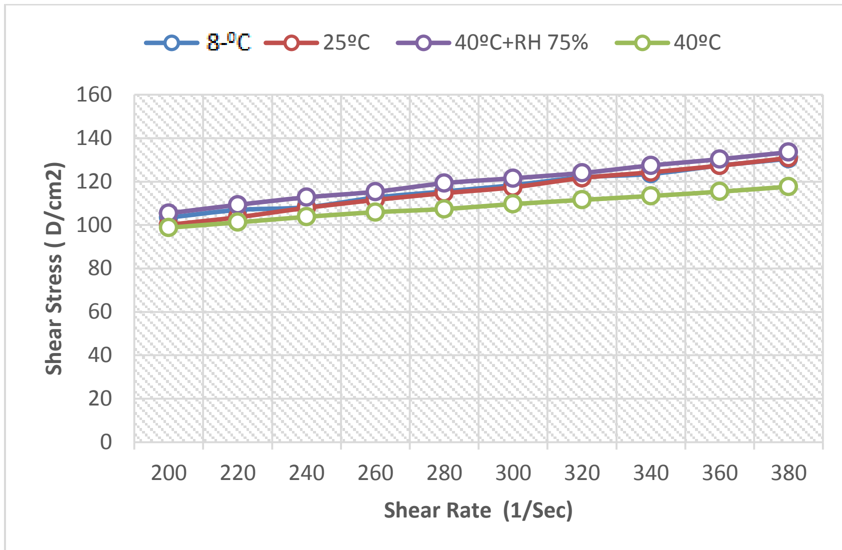


Figure: 4.39.Rheogram of TB at different temperatures after 75 days

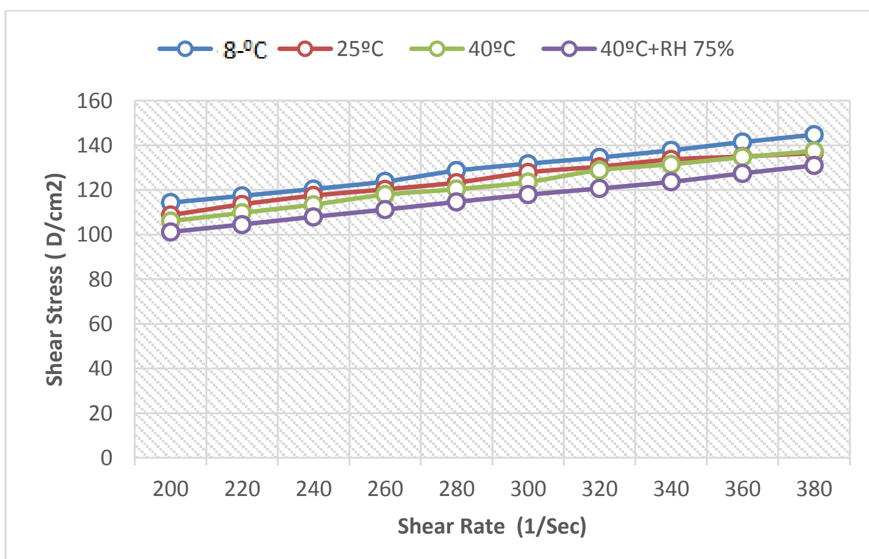


Figure: 4.40. Rheogram of TF at different temperatures after 75 days

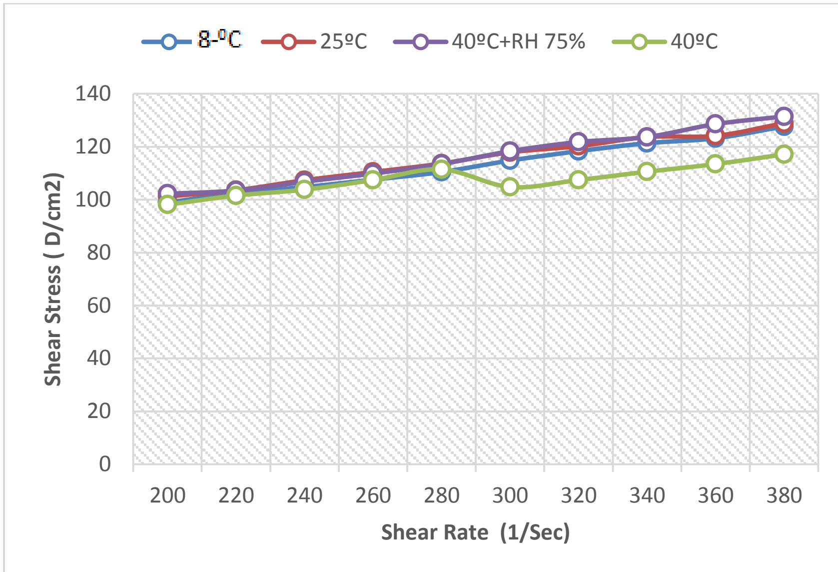


Figure: 4.41. Rheogram of TB at different temperatures after 90 days

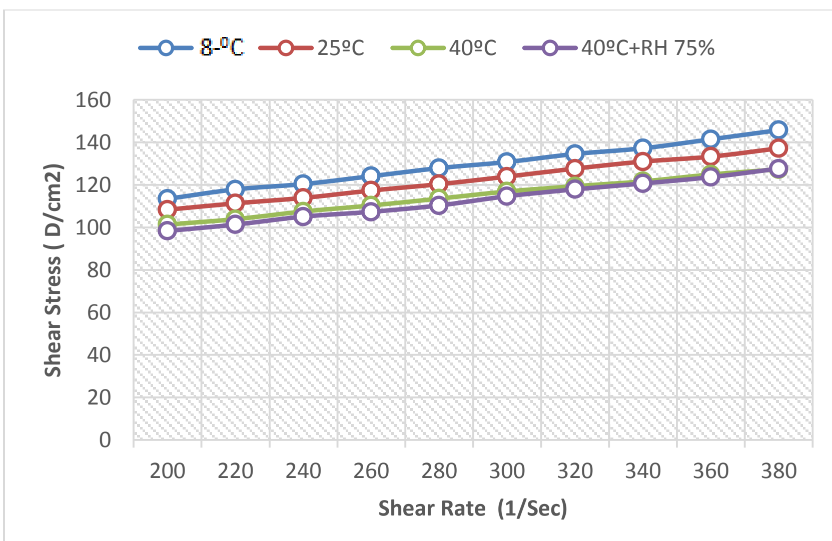


Figure: 4.42.Rheogram of TF at different temperatures after 90 days

4.1.6 Dermatological Tests

Before performing the *in- vivo* evaluation skin irritation assessment (patch test) for Melanin/Erythema was performed on the human volunteer's cheeks. Patch test was performed by all the formulations extracted from the seeds of soybean, grape and tamarind respectively.

4.1.6.1. Emulsion containing crude extract of soybean seeds

4.1.6.1.1. Skin compatibility evaluation (Patch test) for emulsion containing Soybean seeds extract.

To determine the skin irritation assessment, on the first day of study Patch tests were performed on forearms of each volunteer. An area of 5cm × 4cmwas marked on both the forearms of all the volunteers Basic values for erythema and melanin were measured with the help of Mexameter. 1.0 g of base and formulation each were applied to the 5cm X 4cm marked regions separately on each forearm. Surgical dressing was used to cover the marked area of right and left forearm. After 48 hours application of emulsion, it was removed and the forearms were washed with physiological saline and were observed for any skin redness/irritation by a dermatologist. The measurements of erythema and melanin were repeated on both forearms. Scores were recorded for the presence of erythema (skin redness), using a scale for 4 points , from 0 to 3 , where 0 stand for absence , 1 for mild , 2 for moderate and 3 stands for severe erythema respectively. Each volunteer was asked to note their irritation / itching towards the patches and then assign the score from the same scale. Each score with respect to volunteers is given in table **(Table. 4.19**).

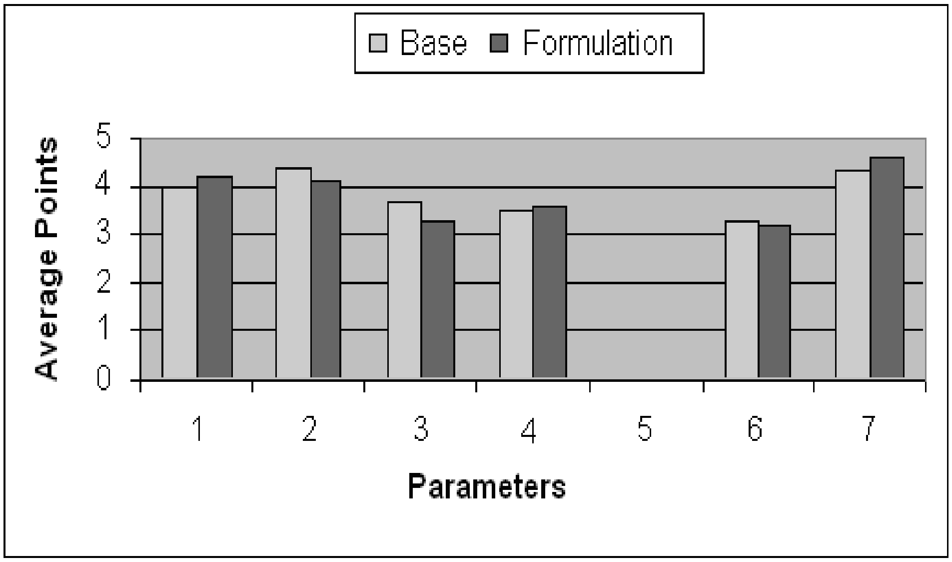
Table 4.19. Score given by 11 volunteers to SB (Soybean base) and SF (Soybean formulation) on the basis of itching and irritation

Score		0	1	2	3
No. volunteers	SB	8	2	2	0
	SF	9	2	1	0

4.1.6.1.2. Panel Test

A questionnaire containing seven questions was prepared and the two copies of this form were given to each volunteer for sensory evaluation of the two creams. Average points were calculated from the points assigned by each volunteer for each question for both of the creams (base and formulation). Average points for the first question, i.e. ease of application of creams were found to be 4.00 and 4.20 for the base and formulation, respectively. This indicated that base and formulation can be easily applied on the skin. Average points regarding spread ability were 4.40 for base and 4.10 for formulation which meant that the formulation spread on skin better than the base. Average points for feel on application were 3.70 for the base and 3.30 for formulation. This indicated that base was felt well on the skin than formulation and the results are shown in **(Figure. 4.43)**. Average points for the sense in long-term application of creams were 3.50 and 3.60 for the base and formulation respectively. This showed that formulation produced more pleasant feeling on application to skin than base. There was no irritation on the skin in both cases i.e. base and formulation, as these were assigned 0.00 point for irritation by all the volunteers. Shine on skin was 3.30 for the base and 3.20 for formulation. This was expected since the base and formulation contained same quantity of paraffin oil. Similarly, the formulation led to more softness of the skin than base as the average point was 4.30 for base and 4.60 for formulation.

It was found from paired sample t-test that there was an insignificant difference between the average points of sensitivity for base and formulation. It was concluded that there was no big variation between base and formulation regarding the sensory evaluation. Both of the creams behaved similarly from the sensory point of view.



s

Figure 4.43. Average Values for Panel Test

Here 1= Ease of application, 2= Spreadbility, 3= Sense just after application 4= Sense in long term, 5= Irritation, 6= Shine on skin, 7= Sense of softness.

4.1.6.1.3. Melanin and erythema

The percent change with respect to time in melanin and erythema following the applications of the Soybean base(SB) and Soybean formulation(SF) on the cheeks of human volunteers has been presented in tables **(Table4.20-4.23)** and represented by figures **(Figure 4.44-4.45)**.

Table 4.20. Percentage of change in values of Skin melanin after application of SB

Volunteer No.	2^{nd} week	4^{th} week	6^{th} week	8^{th} week	10^{th} Week	12^{th} Week
1	-3.36	-4.76	-4.20	2.24	2.24	23.81
2	9.80	12.50	1.22	8.57	42.45	6.94
3	6.76	-0.56	8.73	1.41	-2.54	9.30
4	6.20	13.57	14.34	17.05	17.44	20.16
5	-29.90	5.39	12.75	16.67	2.94	1.96
6	11.59	-3.64	-1.82	-2.73	-3.64	-2.27
7	73.45	60.18	50.88	34.51	51.77	85.84
8	-26.84	-26.84	3.19	10.22	-20.13	-14.38
9	2.67	6.23	1.19	-0.59	-5.64	-9.79
10	-1.32	-16.23	7.89	-5.70	6.14	10.53
11	-10.51	-13.42	-10.31	-5.64	-5.06	-4.28
Mean ± SEM	**3.50±0.33**	**2.95±0.95**	**7.62±1.46**	**6.91±1.50**	**7.82±1.24**	**11.62±1.88**

Table 4.21. Percentage of change in values of Skin melanin after application of SF

Volunteer No.	2nd week	4th week	6th week	8th Week	10th Week	12th Week
1	-2.49	1.39	-1.66	4.43	-3.60	-7.48
2	0.73	-3.66	-0.37	-13.55	7.69	-6.96
3	9.45	0.75	0.78	-7.71	-13.43	-16.42
4	-28.05	-36.88	-43.89	-48.42	-48.42	-50.23
5	-19.08	-22.14	-19.85	-24.05	-35.50	-38.17
6	-13.49	-11.41	-13.90	-18.88	-20.12	-21.58
7	3.44	1.25	6.56	-3.44	-2.19	-17.19
8	-6.93	-1.98	-13.86	-21.45	-30.69	-32.34
9	11.57	5.04	13.65	12.76	-2.97	9.20
10	-2.72	14.01	-14.79	-21.01	-11.28	10.12
11	7.52	-0.61	-0.41	0.61	-1.02	-4.27
Mean ± SEM	**-3.64±0.41**	**-4.93±0.84**	**-7.98±0.71**	**-12.79±0.58**	**-14.68±0.97**	**-15.94±0.56**

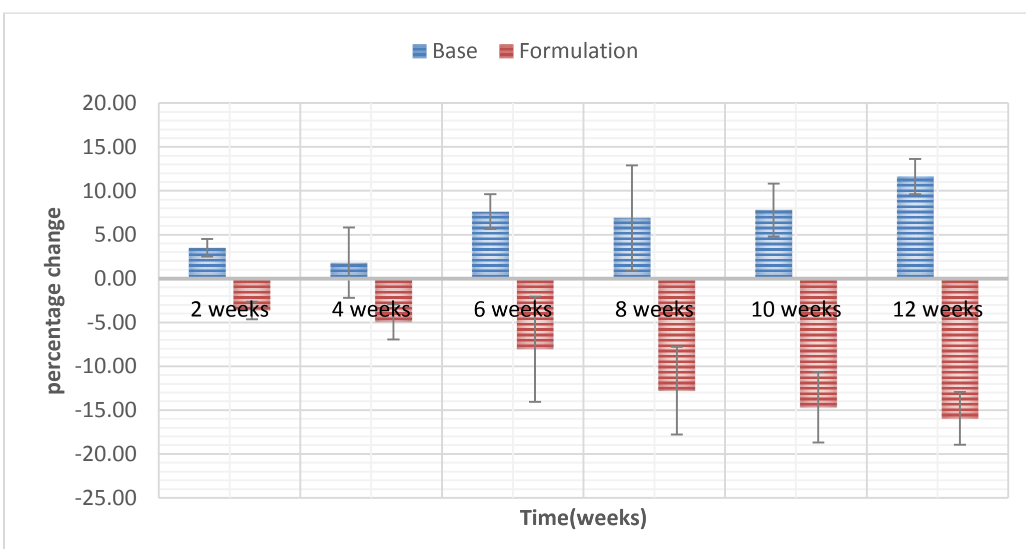


Figure. 4.44. Percentage changes in skin melanin after application of SB and SF.

Table 4.22. Percentage of change in values of Skin erythema after application of SB

Volunteer No.	2nd Week	4th Week	6th week	8th week	10th Week	12th Week
1	-3.09	-4.06	-5.42	-5.61	-4.84	-6.00
2	-5.85	-3.02	-11.29	-10.48	-7.86	-9.07
3	10.95	10.70	11.19	11.44	2.49	0.25
4	12.36	7.32	0.69	1.14	-11.21	-4.12
5	-9.41	1.09	-4.81	-1.97	-8.75	-9.85
6	-0.77	0.00	0.77	1.16	3.08	0.77
7	-17.92	-12.42	-10.79	-6.92	2.24	-2.65
8	4.33	-3.42	-13.90	-39.64	-10.25	-12.30
9	-0.70	-1.39	4.18	4.41	3.25	0.93
10	1.23	0.74	23.21	23.95	22.72	20.25
11	-2.11	-6.95	-8.63	-8.42	-9.47	-10.53
Mean ± SEM	**-1.00±1.33**	**-1.04±1.38**	**-1.35±1.64**	**-2.81±1.50**	**-1.69±1.87**	**-2.94±1.10**

Table 4.23. Percentage of change in values of Skin erythema after application of SF

Volunteer No.	2nd week	4th Week	6th week	8th week	10th week	12th Week
1	-2.18	3.17	0.00	3.17	-0.40	-3.77
2	5.34	-2.99	7.69	10.47	-5.77	-2.99
3	-0.22	-0.22	-1.53	-3.50	-4.60	1.09
4	21.48	4.53	9.07	15.04	-10.26	-3.82
5	-0.47	5.63	5.63	-1.64	1.41	3.99
6	-13.83	-3.37	-9.04	2.30	-5.32	-12.06
7	-10.15	-9.40	-10.71	-26.88	-15.04	-13.53
8	-9.22	4.61	-14.43	-18.44	-17.03	-19.44
9	3.02	-5.10	-4.64	-4.87	8.12	-0.23
10	0.69	-3.94	0.00	0.69	16.20	6.48
11	1.17	-1.17	-1.87	-4.45	-6.09	-7.26
Mean ± SEM	**-0.40±0.80**	**-0.75±0.97**	**-1.80±0.83**	**-2.55±0.92**	**-3.52±0.84**	**-4.69±0.67**

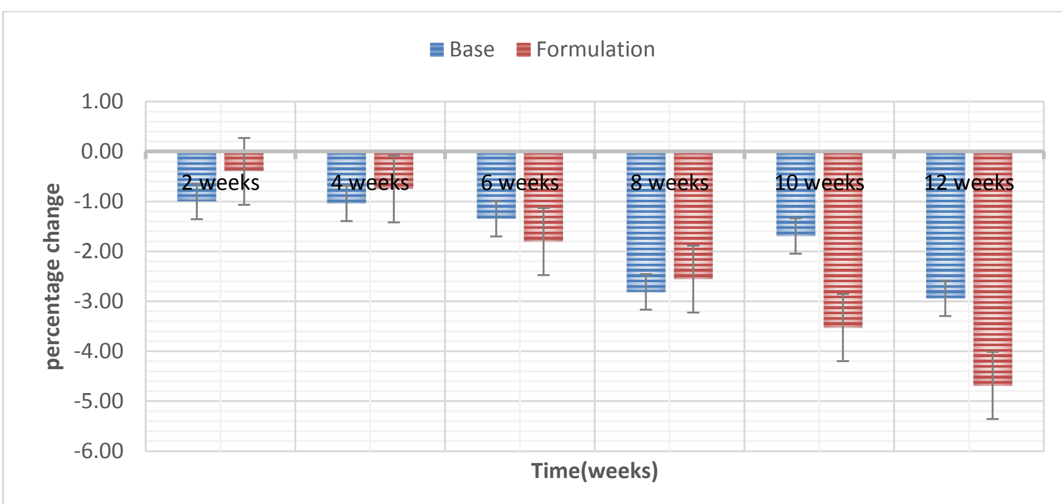


Figure.4.45. Percentage changes in skin erythema after application of SB and SF

4.1.6.1.4. Skin moisture content

The percent changes in the values of skin moisture contents (skin hydration level) with respect to time after application of both Soybean base (SB) and Soybean formulation (SF) are presented in the following tables **(Table 4.24-4.25)** while graphically they are shown by figure **(Figure. 4.46).**

Table 4.24. Percentage of change in values of Skin Moisture after application of SB

Volunteer No.	2nd Week	4th week	6th week	8th week	10th week	12th Week
1	-3.80	-3.42	-0.76	0.76	5.70	1.52
2	3.82	-3.18	3.50	0.32	3.82	5.73
3	-0.31	0.31	-3.37	0.92	1.84	5.83
4	-2.41	3.92	7.23	9.64	3.92	6.02
5	5.06	7.30	1.69	6.18	10.67	3.09
6	-5.22	-1.49	-5.60	0.00	-3.73	-2.61
7	-3.01	-0.30	3.01	2.71	2.11	15.06
8	1.49	5.95	4.76	5.95	9.82	14.58
9	-1.35	1.35	2.69	4.93	-0.90	9.42
10	-5.01	-7.52	-9.47	-15.32	-13.09	-14.76
11	0.89	3.13	10.49	0.89	-6.03	-2.23
Mean ± SEM	**-0.90±1.04**	**0.55±1.33**	**1.29±1.74**	**1.54±1.93**	**1.29±2.10**	**3.79±2.55**

Table 4.25. Percentage of change in values of Skin Moisture after application of SF

Volunteer No.	2nd Week	4th week	6th week	8th week	10th week	12th Week
1	-2.95	-1.48	-0.74	0.00	4.80	2.21
2	23.62	9.20	11.96	19.33	39.88	35.58
3	13.71	1.25	5.30	18.07	44.24	42.68
4	10.72	40.58	36.81	34.78	36.52	39.71
5	12.54	17.38	2.28	22.79	14.53	3.13
6	7.69	-6.96	9.16	15.02	25.27	30.40
7	-6.21	15.22	3.11	23.29	27.64	31.68
8	11.75	14.92	8.25	11.75	10.48	16.83
9	-0.93	11.57	4.63	15.28	-6.94	0.00
10	-5.52	5.52	-21.55	1.66	-8.29	2.76
11	-5.29	0.69	1.61	8.28	11.72	18.62
Mean ± SEM	**5.37±3.03**	**9.81±3.88**	**5.53±4.10**	**15.48±3.03**	**18.17±5.45**	**20.33±4.96**

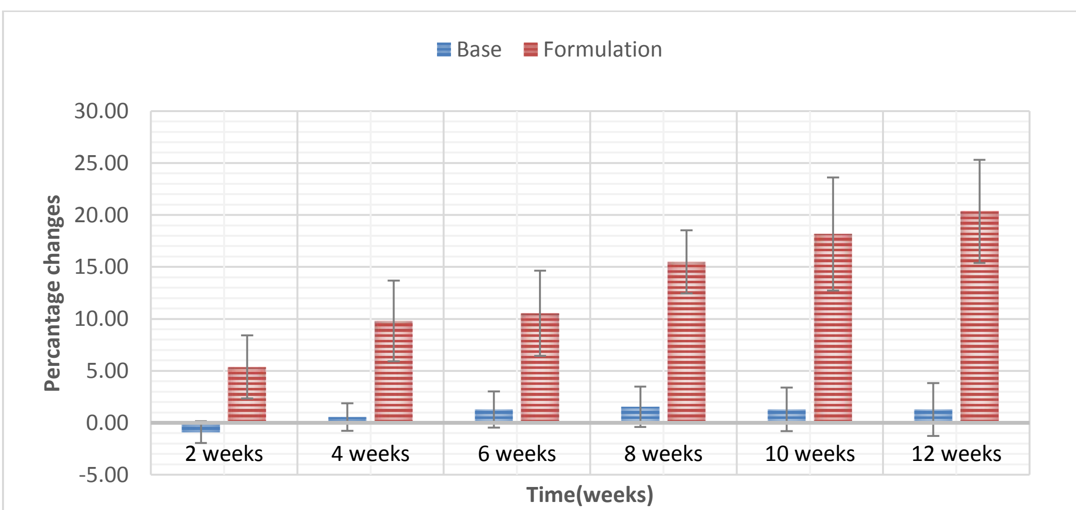


Figure.4.46. Percentage changes in skin moisture contents after application of SB and SF

4.1.6.1.5.Skin Elasticity

After 12 weeks study the percent changes in the values of skin elasticity following applications of Soybean base(SB) and Soybean formulation (SF) have been given in tables **(Table 4.26, 4.27)** and represented by figure **(Figure 4.47).**

Table 4.26. Percentage of change in values of Skin Elasticity after application of SB

Volunteer No.	2nd Week	4th week	6th week	8th week	10th week	12th Week
1	-3.17	7.94	-6.35	-3.17	7.94	3.17
2	7.02	5.26	-10.53	7.02	-1.75	-3.51
3	1.52	1.52	-1.52	-13.64	-9.09	4.55
4	4.76	-19.05	11.11	6.35	-11.11	6.35
5	-1.47	8.82	2.94	-4.41	4.41	7.35
6	-1.30	-11.69	0.00	5.19	-9.09	-9.09
7	14.55	9.09	3.64	23.64	1.82	10.91
8	-14.49	4.35	1.45	-5.80	7.25	1.45
9	11.29	4.84	12.90	8.06	17.74	-4.84
10	-6.15	7.69	-3.08	-6.15	0.00	1.54
11	4.35	-4.35	7.25	-1.45	7.25	-2.90
Mean ± SEM	**1.54±1.79**	**1.31±1.47**	**1.62±1.14**	**1.42±2.03**	**1.40±1.66**	**1.36±1.20**

Table 4.27. Percentage of change in values of Skin Elasticity after application of SF

Volunteer No.	2nd Week	4th week	6th week	8th week	10th week	12th Week
1	17.86	3.57	12.50	16.07	23.21	33.93
2	4.00	28.00	8.00	24.00	30.00	44.00
3	3.03	-6.06	-1.52	6.06	18.18	13.64
4	-7.58	-12.12	1.52	-1.52	18.18	13.64
5	6.15	16.92	23.08	-4.62	13.85	9.23
6	29.51	45.90	37.70	39.34	24.59	14.75
7	-14.67	4.00	-10.67	9.33	6.67	17.33
8	7.14	-4.29	0.00	-2.86	7.14	5.71
9	7.25	0.00	7.25	-5.80	-13.04	1.45
10	3.23	8.06	17.74	9.68	8.06	9.68
11	-3.70	-12.35	-8.64	-11.11	-19.75	-6.17
Mean ± SEM	**4.75±2.58**	**6.51±2.37**	**7.91±3.32**	**7.14±3.51**	**10.64±3.65**	**14.29±3.25**

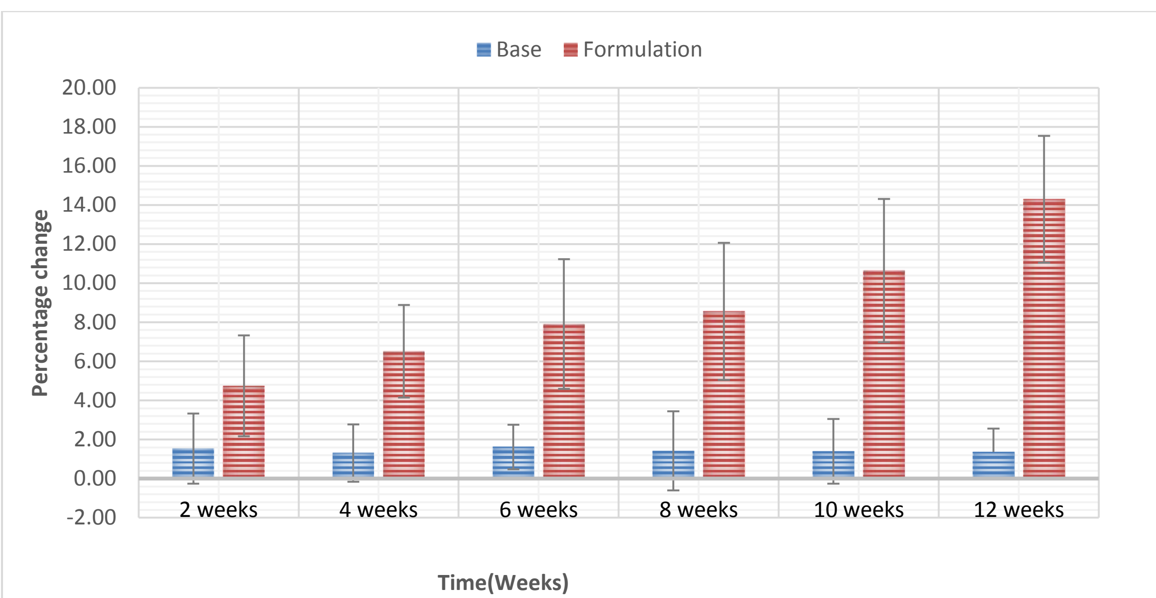


Figure .4.47. Percentage changes in skin elasticity after application of SB and SF.

4.1.6.1.6. Skin sebum content

The percentage of change in the measured skin sebum values after applications of Soybean base (SB) and Soybean formulation(SF) have been given in tables **(Table 4.28, 4.29)** and represented by figure **(Figure. 4.48)**.

Table 4.28. Percentage of change in values of Skin Sebum after application of SB

Volunteer No.	2nd Week	4th week	6th week	8th week	10th Week	12th Week
1	40.54	83.78	105.41	156.76	232.43	283.78
2	1.16	12.79	5.81	19.77	3.49	11.63
3	21.84	12.64	16.09	-26.44	1.15	21.84
4	5.10	-5.10	57.14	62.24	8.16	25.51
5	8.47	-16.95	10.17	-23.73	-8.47	-50.85
6	16.05	9.88	122.22	-32.10	9.88	3.70
7	-4.03	-16.94	-17.74	4.84	6.45	0.00
8	-4.79	-12.77	-21.81	-11.70	-6.38	4.26
9	40.30	11.94	-7.46	80.60	176.12	113.43
10	-23.58	-41.06	-43.50	-48.78	-40.65	-24.80
11	-1.43	54.29	-15.00	54.29	1.43	17.86
Mean ± SEM	**9.06±3.89**	**8.41±4.55**	**19.21±5.16**	**21.43±6.64**	**34.87±7.93**	**36.94±8.56**

Table 4.29. Percentage of change in values of Skin Sebum after application of SF

Volunteer No.	2nd Week	4th week	6th week	8th week	10th Week	12th Week
1	2.86	8.57	-2.86	-17.14	-20.00	-25.71
2	54.05	56.76	2.70	-8.11	-10.81	-8.11
3	5.13	5.12	-7.69	-7.65	2.56	-10.26
4	-19.77	-9.08	-5.81	-17.44	-12.79	-11.63
5	-9.09	-13.64	-4.55	-13.64	-22.73	-13.64
6	-0.97	-6.80	-3.88	-4.85	-1.94	-6.80
7	3.45	-5.17	-3.45	-6.90	-1.72	-6.90
8	1.63	-7.32	-6.50	-4.07	-8.94	-4.88
9	-0.96	-1.92	-21.15	-1.44	-3.37	-4.81
10	6.10	-6.10	-8.13	-4.47	-6.10	-6.91
11	-7.34	-6.42	-3.67	-9.17	-12.84	-11.01
Mean ± SEM	**3.19±4.59**	**1.27±5.34**	**-5.91±2.77**	**-8.63±1.79**	**-8.97±2.38**	**-10.06±2.79**

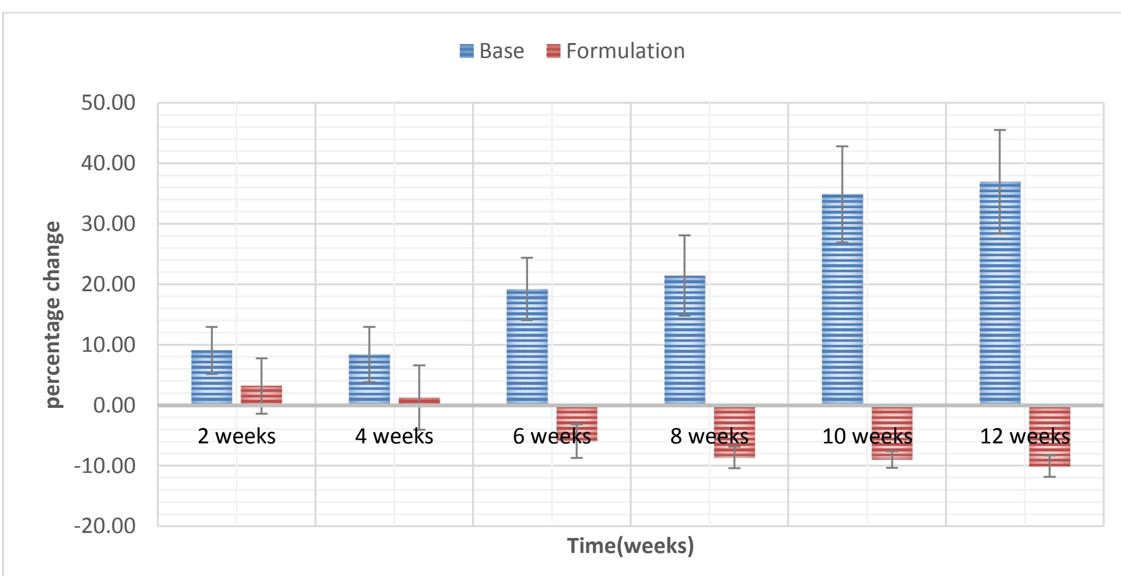


Figure 4.48. Percentage changes in skin sebum after application of SB and SF.

4.1.6.1.7. Surface Evaluation of Living Skin (SELS)

Visioscan VC 98/software SELS 2000 (Courage and Khazaka GmbH) is used for the measurement of SELS parameters SE*w* , SE*sc*, SE*sm* and SE*r* before the application of emulsions containing soybean extract and then at 1st, 2nd and 3rd months of the study duration are given in (**Table. 4.30)**. An example of typical and 3D image taken by SELS 2000 software of a volunteer facial skin at base line and after 3 months treatment has been shown in figures (**Figure. 4.49-4.52).**

Table 4.30 SELS parameters values(Mean±SD)

Parameters	Emulsion	0 hour	1month	2 month	3 month
SEr	**Base**	4.05±0.087	4.04±0.07	4.04±0.090	4.04±0.086
	Formulation	4.04±0.086	4.00±0.084	3.95±0.084	3.82±0.081
SEsc	**Base**	1.74±0.050	1.74±0.050	1.73±0.051	1.73±0.050
	Formulation	1.73±0.049	1.70±0.050	1.68±0.054	1.66±0.055
SEsm	**Base**	108.99±4.91	109.98±4.91	108.64±4.89	107.82± 4.92
	Formulation	102.01±4.77	100.72±4.81	98.72±4.66	92.03± 4.23
Sew	**Base**	71.84±1.54	70.83±1.55	69.73±1.54	69.83±2.00
	Formulation	67.74±1.52	71.93±1.53	69.96±1.65	68.51±1.64

SEr ;skin roughness, , SEw ;skin wrinkles, , SEsm; skin smoothness SEsc ;skin scaliness

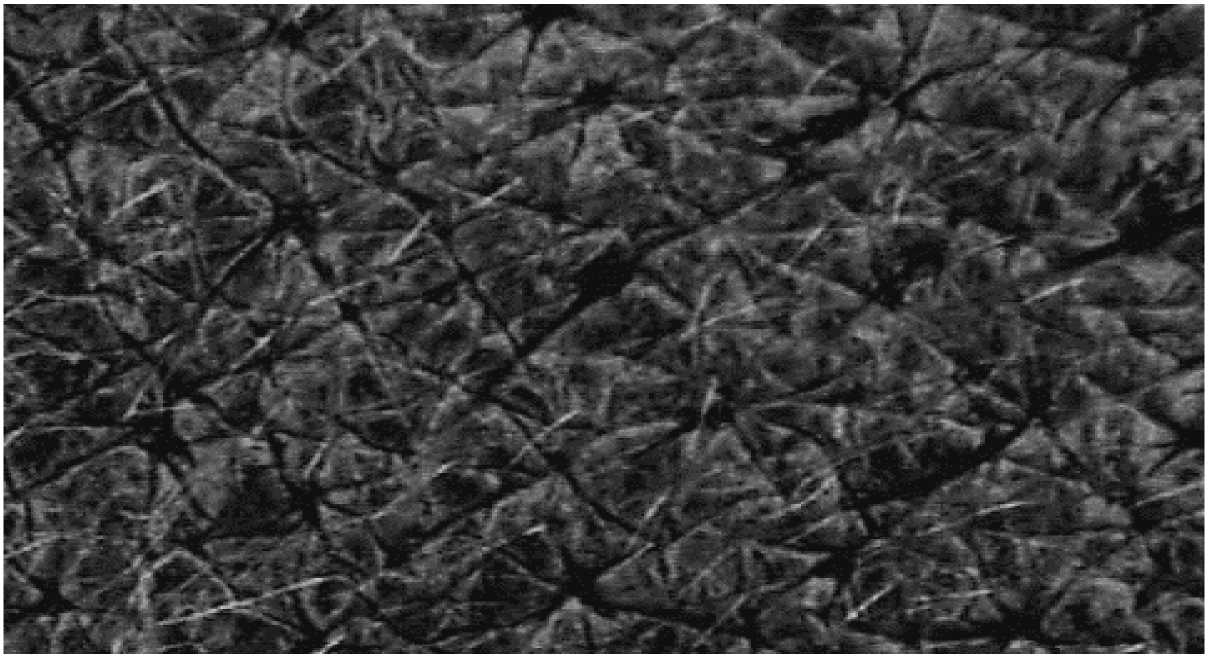


Figure 4.49. An image of skin taken by Visioscan® before application of SF

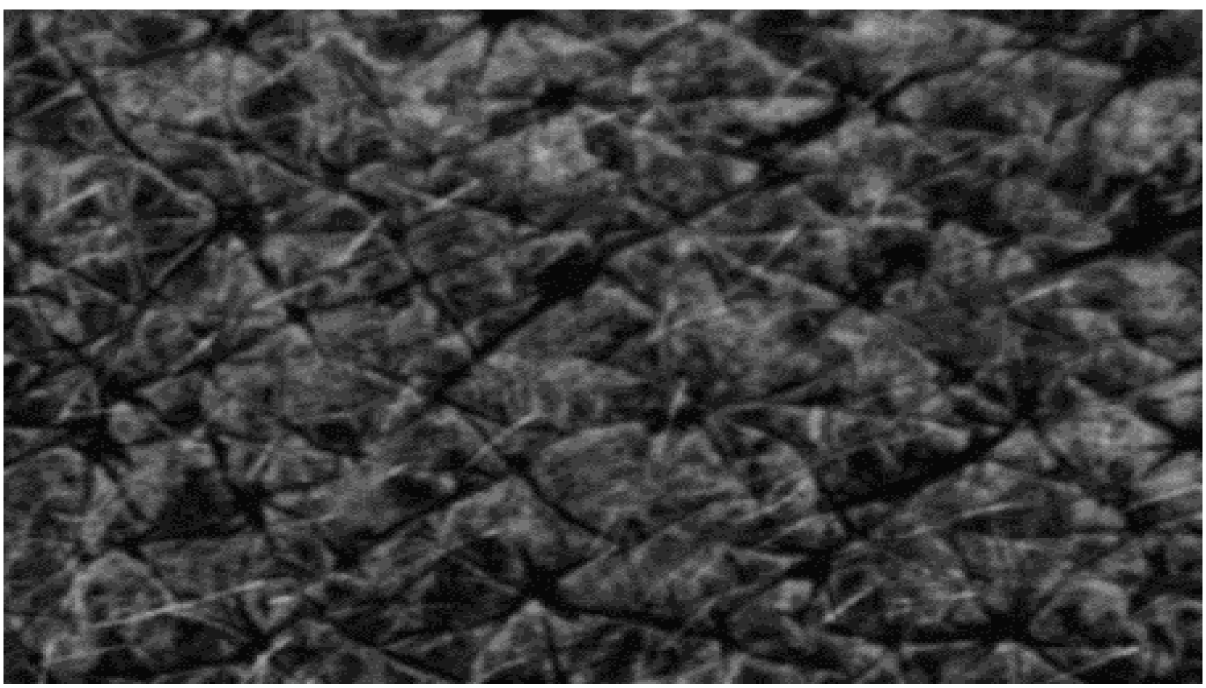


Figure 4.50. An image of skin taken by Visioscan® after 3 months of application of SF

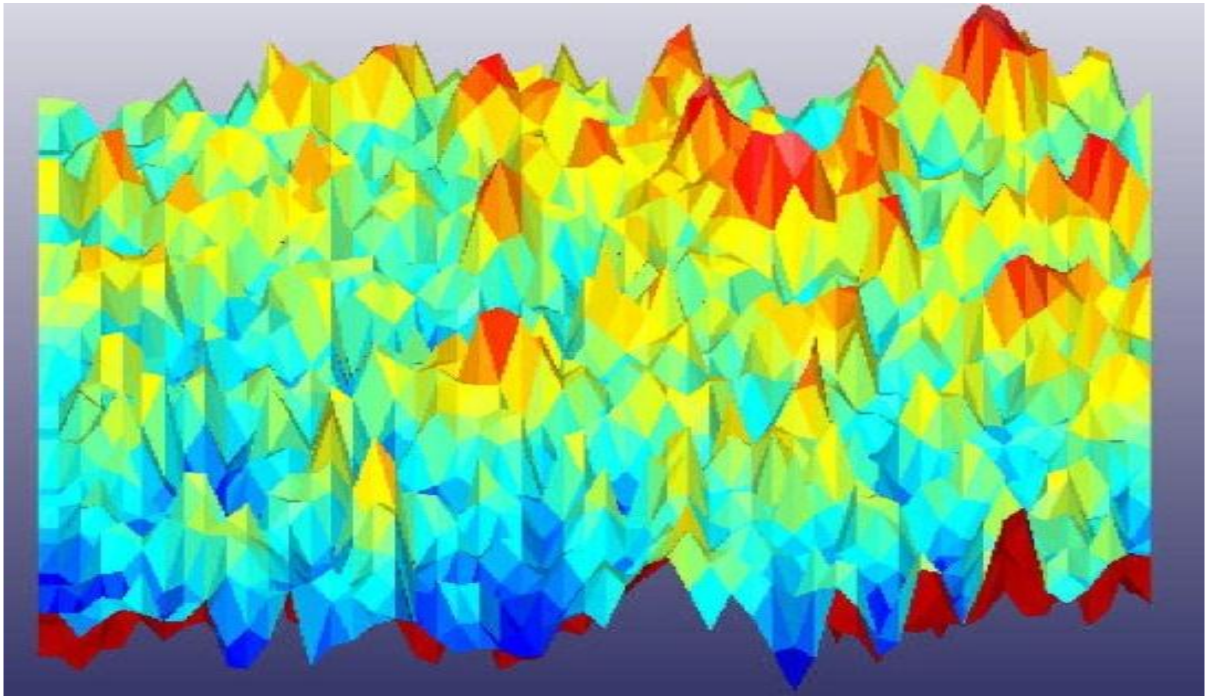


Figure 4.51. 3D image of skin taken by Visioscan® before application of SF

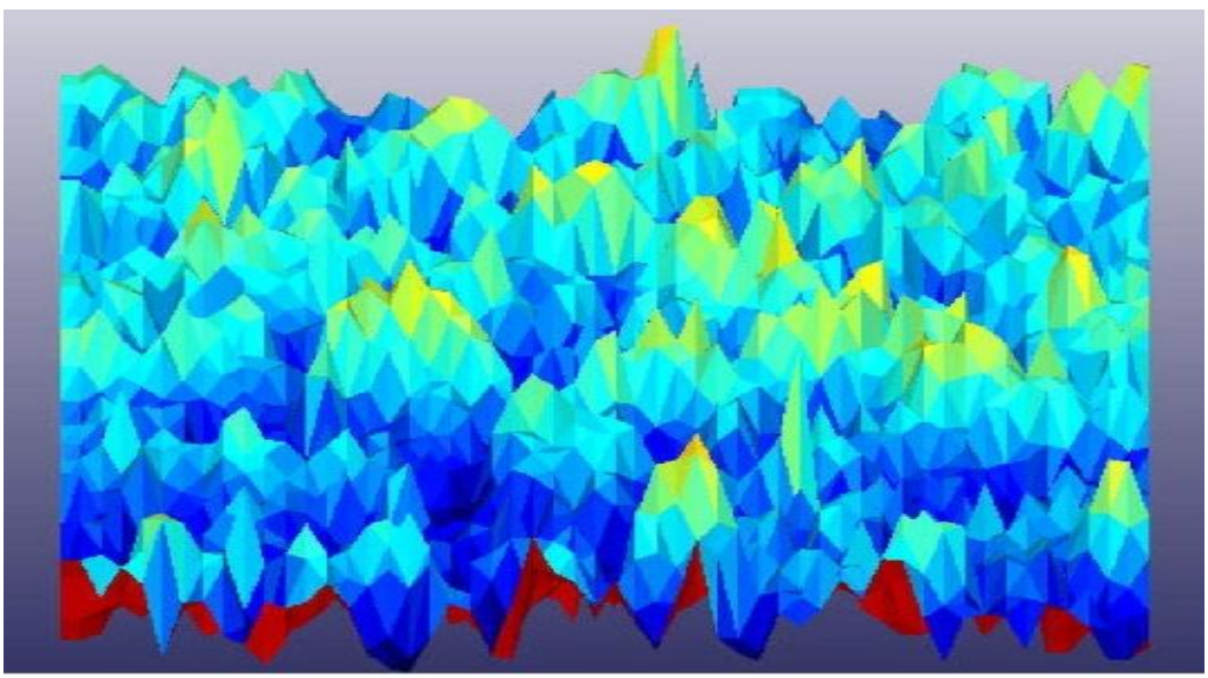


Figure 4.52. 3D image of skin taken by Visioscan® after 3 months of application of SF

4.1.6.2.Emulsion containing crude extract of Grape seeds

4.1.6.2.1. Skin sensitivity Test (Patch Test)

Patch test for emulsion containing grape seeds extract was performed with same procedure as adopted with emulsion containing soybean seeds extract. Each score with respect to volunteers is given in **(Table. 4.31)**.

Table: 4.31. Score given by volunteers to GB (Grape seeds base) and GF (Grape seeds formulation) on the basis of itching and irritation

Score		0	1	2	3
No. volunteers	GB	8	2	2	0
	GF	9	2	1	0

4.1.6.2.2. Panel test

Same procedure for Panel test for emulsion containing Grape seeds extract was performed as adopted with emulsion containing Soybean seeds extract. Paired sample t-test was applied and it was found that there was an insignificant difference between the average points of sensitivity for Base and Formulation. Both the emulsions have similar performance from the sensory point of view **(Table. 4.32)**.

Table: 4.32. Average Values ± SEM for Panel Test by 11 Volunteers for GB and SF

	Average points for Base ± SEM	Average points for Formulation ± SEM
Ease of application	4.12 ± 0.05	4.12± 0.10
Spreadability	4.20 ± 0.04	4.24± 0.06
Sense just after application	4.00± 0.06	4.01 ± 0.09
Sense in long term	4.15 ± 0.07	4.15± 0.08
Irritation	0.00 ± 0.00	0.00 ± 0.000
Shine on skin	4.10± 0.08	4.22± 0.03
Sense of softness	4.30 ± 0.05	4.38± 0.06

4.1.6.2.3. Melanin and erythema

The percent change with respect to time in melanin and erythema following the applications of the Grape seeds base(GB) and Grape seeds formulation (GF) on the cheeks of human volunteers has been presented in tables **(Table 4.33-4.36) and** represented by figures **(Figure. 4.53-4.54)**.

Table 4.33. Percentage of change in values of Skin Melanin after application of GB

Volunteer No.	2nd Week	4th week	6th week	8th Week	10th Week	12th Week
1	3.33	2.42	2.12	7.27	9.70	3.03
2	1.90	4.18	4.94	0.38	-2.66	-0.38
3	0.75	3.01	2.01	2.76	3.76	5.01
4	2.09	-1.16	-1.86	-2.55	-4.64	1.62
5	-2.29	-3.43	2.29	1.71	2.86	4.57
6	0.80	1.87	-1.33	-2.40	-0.53	2.67
7	3.24	2.65	3.83	0.88	-1.47	1.77
8	1.56	3.12	4.36	6.85	8.72	7.17
9	3.98	4.68	2.81	1.41	-0.70	3.04
10	2.03	3.54	4.81	5.57	6.33	7.85
11	2.16	0.81	-2.97	3.24	4.32	2.43
Mean ± SEM	**1.78±0.51**	**1.97±0.73**	**1.91±0.84**	**2.28±1.00**	**2.33±1.43**	**3.53±0.74**

Table 4.34. Percentage of change in values of Skin Melanin after application of GF

Volunteer No.	2nd Week	4th week	6th week	8th Week	10th Week	12th Week
1	-2.51	1.39	-1.67	4.46	5.29	-7.52
2	-2.58	-3.69	-0.37	-13.65	7.38	-6.64
3	8.96	0.50	0.00	-7.96	-14.43	-15.42
4	-28.18	-37.05	-44.09	-48.41	-49.32	-50.45
5	-19.54	-22.22	-20.31	-24.52	-36.02	-38.70
6	-13.54	-13.54	-13.96	-18.96	-15.83	-21.25
7	3.14	1.26	6.60	-3.46	-2.52	-26.10
8	-4.98	-1.99	-13.95	-21.59	-30.90	-32.56
9	11.64	5.07	13.73	12.84	-2.99	9.25
10	-2.75	14.12	-14.90	-21.18	-11.37	10.20
11	7.54	-0.61	-0.41	0.61	-1.02	-4.28
Mean ± SEM	**-3.89±4.28**	**-5.16±4.76**	**-8.12±5.09**	**-12.89±3.73**	**-13.79±5.47**	**-16.68±5.83**

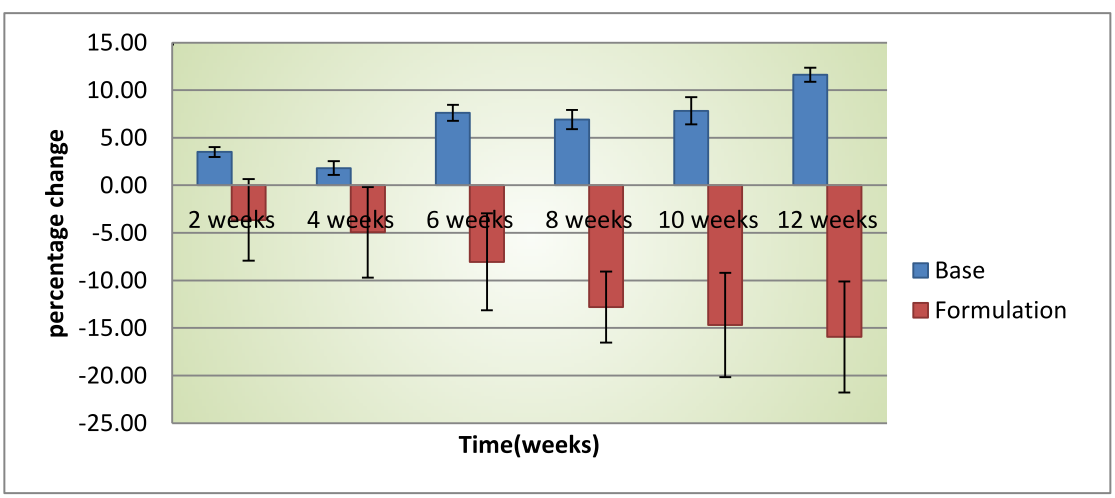


Figure 4.53. Percentage changes in skin melanin contents after application of GB and GF

Table 4.35. Percentage of change in values of Skin Erythema after application of GB

Volunteer No.	2nd week	4th week	6th week	8th Week	10th Week	12th Week
1	-0.65	-1.94	-3.55	-4.19	-5.48	-8.06
2	-1.77	-2.88	-3.32	-2.43	-3.10	-6.86
3	-1.71	-2.00	-4.57	-7.71	-8.86	-10.00
4	-0.71	-1.42	-3.08	-1.66	-1.42	-2.61
5	-3.49	-4.07	-3.20	-5.52	-6.98	-7.56
6	-0.69	0.23	-1.61	-1.83	-0.46	4.82
7	0.78	2.52	3.30	3.88	2.91	7.57
8	-2.22	-2.82	-4.03	-4.23	-2.82	-3.63
9	-0.80	-1.40	0.60	2.00	1.00	4.40
10	0.94	0.47	-1.89	-0.71	0.24	1.65
11	1.50	2.24	2.74	4.24	3.49	2.49
Mean ± SEM	**-0.80±0.45**	**-1.01±0.64**	**-1.69±0.82**	**-1.65±1.15**	**-1.95±1.20**	**-1.62±1.84**

Table 4.36. Percentage of change in values of Skin Erythema after application of GF

Volunteer No.	2nd week	4th week	6th week	8th Week	10th Week	12th Week
1	1.99	2.65	-0.99	-2.32	-4.97	-6.95
2	0.65	1.51	-0.65	-2.15	-3.01	-4.09
3	-1.46	-0.29	-1.75	-4.37	-5.25	-7.87
4	-0.46	-1.61	-3.22	0.23	-3.45	-4.83
5	-1.23	-4.60	-5.52	-4.29	-5.21	-7.36
6	1.98	-0.22	-0.66	-1.98	-2.64	-4.63
7	0.79	-0.59	-1.38	-1.98	-3.56	-4.15
8	-2.58	-2.78	-3.77	-5.75	-8.33	-8.73
9	-1.44	-2.27	-4.95	-5.57	-4.33	-3.51
10	2.50	3.25	5.00	6.75	1.00	-1.25
11	-6.60	-8.38	-4.31	-6.85	-3.81	-10.66
Mean ± SEM	**-0.53±0.79**	**-1.21±1.01**	**-2.02±0.88**	**-2.57±1.13**	**-3.96±0.68**	**-5.82±0.82**

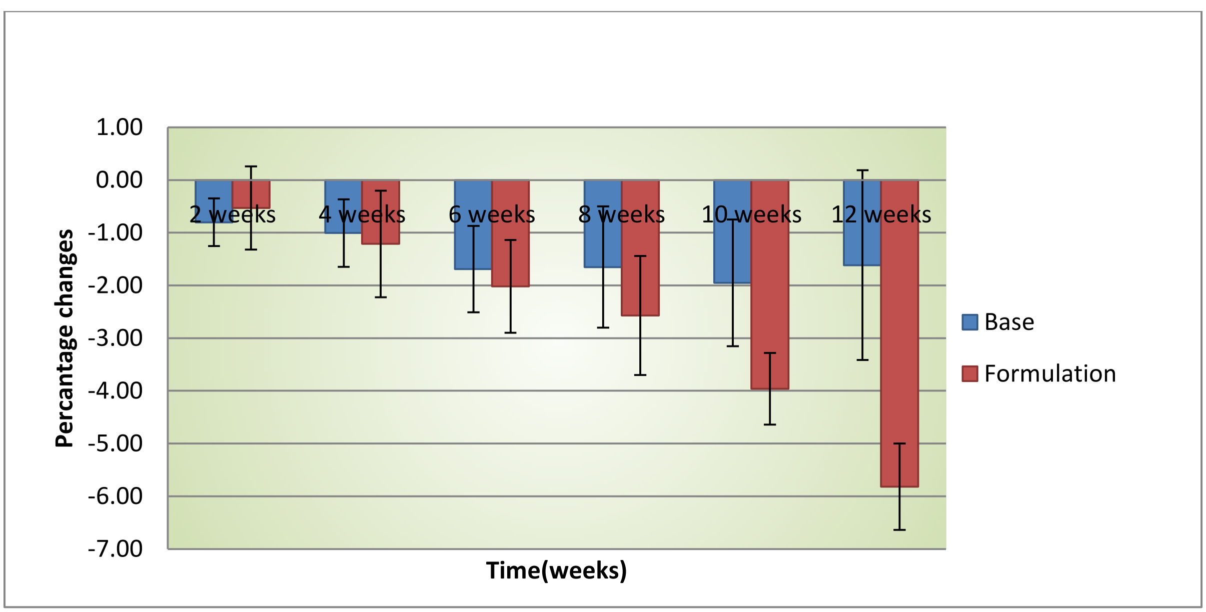


Figure 4.54. Percentage changes in skin erythema contents after application of GB and GF

4.1.6.2.4. Skin moisture content

The percent changes in the values of skin moisture contents (skin hydration level) with respect to time after application of both Grape seeds base (GB) and Grape seeds formulation (GF) are presented in the following tables **(Table. 4.37, 4.38)** while graphically they are shown by figure **(Figure. 4.55).**

Table 4.37. Percentage of change in values of skin moisture after application of GB

Volunteer No.	2nd week	4th Week	6th week	8th week	10th Week	12th Week
1	-3.67	-3.39	1.98	2.54	-6.21	-7.91
2	-4.28	-6.25	0.33	3.29	4.93	5.92
3	2.10	10.84	3.50	11.19	13.99	15.03
4	-4.20	-7.00	-2.80	0.28	1.40	1.96
5	-4.15	2.90	-11.62	4.98	6.64	8.30
6	4.94	-26.24	-6.08	-3.42	-19.77	-4.18
7	-3.66	-10.98	0.91	3.05	-7.01	2.13
8	-1.09	6.91	8.36	10.55	11.27	20.73
9	4.09	0.94	4.40	-10.38	-1.26	2.52
10	-5.08	5.33	3.63	4.60	0.97	6.30
11	-4.01	-0.86	1.43	4.30	1.43	-4.87
Mean ± SEM	**-1.73±1.11**	**-2.53±3.08**	**0.37±1.65**	**2.82±1.81**	**0.58±2.82**	**4.17±2.57**

Table: 4.38. Percentage of change in values of skin moisture after application of GF

Volunteer No.	2nd week	4th week	6th week	8th week	10th Week	12th Week
1	6.80	0.57	1.70	9.07	17.56	11.05
2	3.49	9.52	15.24	20.95	27.62	22.86
3	5.94	4.29	7.92	19.47	23.10	26.73
4	2.33	9.01	5.81	4.07	4.94	9.88
5	-3.95	19.37	28.06	32.41	41.50	35.57
6	3.64	8.00	17.45	29.45	21.82	29.45
7	4.59	13.43	19.43	21.91	24.73	29.68
8	3.58	8.66	5.97	19.70	5.67	11.64
9	14.09	27.49	-2.75	14.43	4.47	10.65
10	-5.92	-2.30	6.25	17.11	10.20	17.11
11	-2.28	10.13	21.01	15.44	22.28	18.23
Mean ± SEM	**2.94±1.67**	**9.83±2.50**	**11.46±2.83**	**18.55±2.45**	**18.54±3.46**	**20.26±2.75**

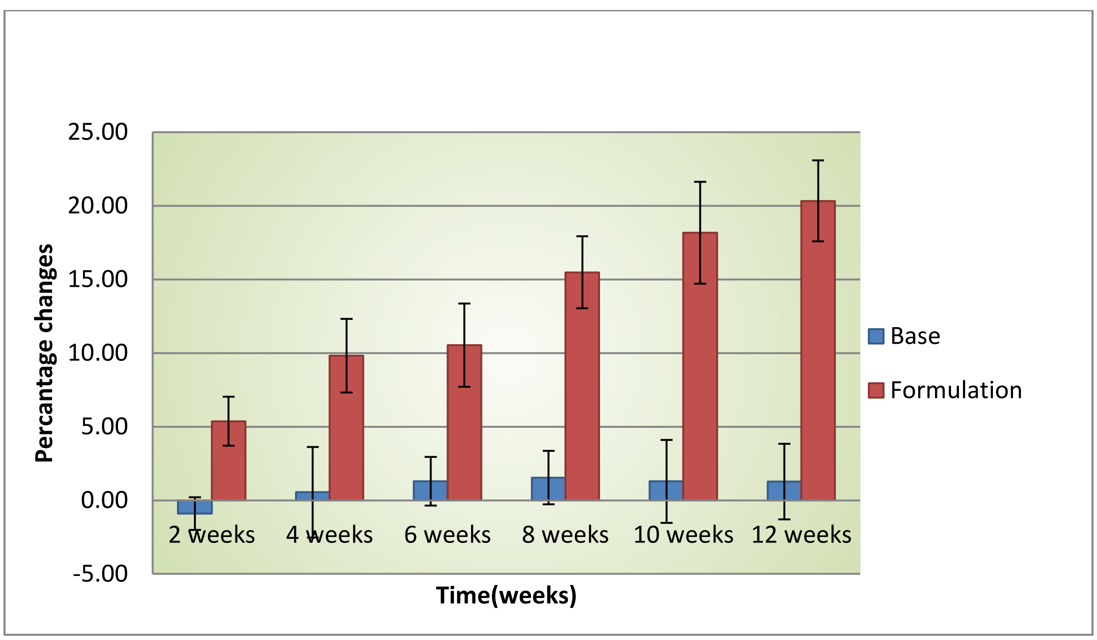


Figure 4.55. Percentage changes in skin moisture contents after application of GB and GF

4.1.6.2.5. Skin Elasticity

After 12 weeks study the percent changes in the values of skin elasticity following applications of grape seeds base(GB) and grape seeds formulation (GF) have been given in tables **(Table. 4.39, 4.40)** and represented by figures **(Figure. 4.56).**

Table: 4.39. Percentage of change in values of skin elasticity after application of GB

Volunteer No.	2nd week	4th week	6th week	8th week	10th Week	12th Week
1	-4.62	7.69	-6.15	-3.08	7.69	3.08
2	8.77	7.02	-8.77	8.77	0.00	-1.75
3	5.88	0.00	-2.94	-14.71	-8.82	2.94
4	6.25	-18.75	9.38	6.25	-10.94	6.25
5	-2.86	7.14	1.43	-5.71	2.86	5.71
6	-2.53	-12.66	-1.27	3.80	-10.13	-5.06
7	10.53	7.02	1.75	19.30	0.00	7.02
8	-5.48	1.37	-2.74	-8.22	2.74	-4.11
9	-10.94	4.69	10.94	6.25	15.63	-6.25
10	-4.48	5.97	-4.48	-7.46	0.00	0.00
11	2.82	-5.63	5.63	-2.82	5.63	-4.23
Mean ± SEM	**0.30±0.08**	**0.35±1.72**	**0.25±1.90**	**0.22±1.92**	**0.42±1.48**	**0.33±1.43**

Table 4.40. Percentage of change in values of skin elasticity after application of GF

Volunteer No.	2nd week	4th week	6th week	8th week	10th Week	12th Week
1	18.52	3.70	12.96	-1.85	24.07	35.19
2	4.17	29.17	8.33	25.00	31.25	45.83
3	3.13	-6.25	-1.56	6.25	18.75	14.06
4	-7.81	-12.50	1.56	-4.69	18.75	14.06
5	6.35	17.46	23.81	-4.76	14.29	9.52
6	30.51	47.46	38.98	40.68	25.42	15.25
7	4.11	0.00	-10.96	9.59	6.85	17.81
8	7.35	-13.24	0.00	-2.94	7.35	5.88
9	7.46	-5.97	-4.48	-5.97	-13.43	1.49
10	3.33	-5.00	18.33	16.67	8.33	10.00
11	-3.80	-12.66	-8.86	-11.39	-20.25	-6.33
Mean ± SEM	**6.67±0.12**	**3.83±1.93**	**7.10±1.61**	**6.05±1.78**	**11.03±1.81**	**14.80±1.42**

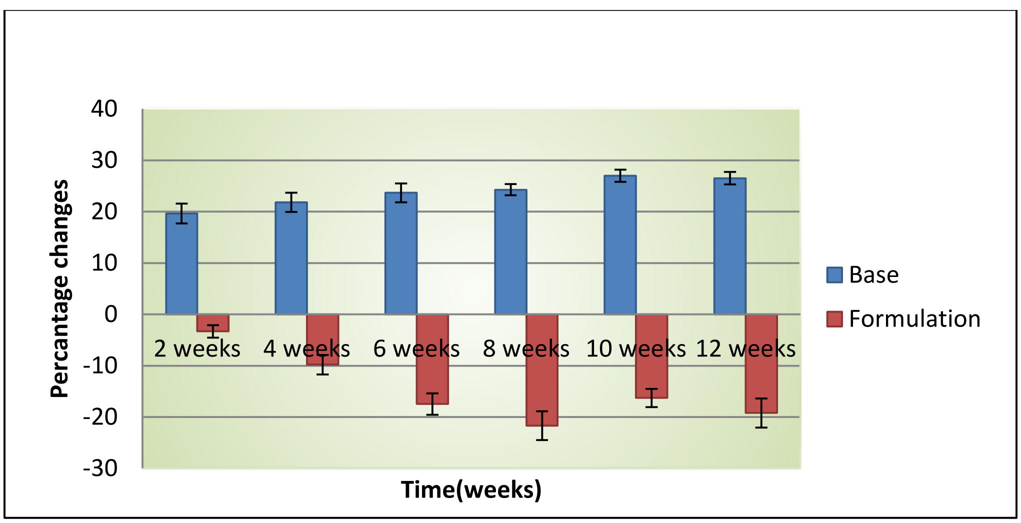


Figure 4.56. Percentage changes in skin elasticity after application of GB and GF

4.1.6.2.6. Skin sebum content

The percentage of change in the measured skin sebum values after applications of Grape seeds base (GB) and grape seeds formulation(GF) have been given in tables **(Table. 4.41, 4.42)** and represented by figures **(Figure. 4.57)**.

Table 4.41. Percentage of change in values of skin sebum after application of GB

Volunteer No.	2nd week	4th Week	6th week	8th week	10th Week	12th Week
1	30.00	5.00	17.50	12.50	10.00	30.00
2	104.65	97.67	100.00	106.98	113.95	125.58
3	20.69	17.24	12.64	16.09	6.90	5.75
4	5.26	8.77	10.53	1.75	31.58	5.26
5	3.17	7.94	4.76	1.59	15.87	9.52
6	1.92	23.08	15.38	13.46	30.77	17.31
7	9.09	15.15	22.73	1.52	16.67	15.15
8	20.00	13.85	18.46	30.77	29.23	20.00
9	10.53	28.07	24.56	40.35	10.53	15.79
10	5.00	6.67	13.33	20.00	6.67	18.33
11	5.48	16.44	20.55	21.92	24.66	28.77
Mean ± SEM	**19.62±1.93**	**21.81±1.89**	**23.68±1.84**	**24.27±1.08**	**26.98±1.17**	**26.50±1.22**

Table 4.42. Percentage of change in values of skin sebum after application of GF

Volunteer No.	2nd week	4th week	6th week	8th week	10th Week	12th Week
1	-9.33	-15.29	-17.67	-25.19	-23.14	-38.29
2	-3.00	-9.00	-13.00	-29.00	-27.00	-23.00
3	-15.81	-19.52	-23.22	-31.74	-24.33	-23.44
4	-6.14	-5.00	8.14	-10.00	-5.29	-13.29
5	-17.98	2.89	-25.53	-33.83	-29.49	-25.42
6	8.02	-2.00	-9.77	-4.51	2.75	-6.79
7	-15.29	-22.43	-26.00	-28.29	-21.00	-25.43
8	-3.78	-23.22	-34.33	-34.33	-14.89	-13.22
9	-4.45	-11.34	-17.09	-22.84	-26.29	-13.64
10	-13.28	9.77	-18.54	-4.51	8.02	-9.77
11	-6.61	-12.55	-15.19	-14.53	-18.49	-19.12
Mean ± SEM	**-3.33±1.2**	**-9.79±0.9**	**-17.47±2.1**	**-21.71±1.8**	**-16.29± 1.83**	**-19.22± 1.73**

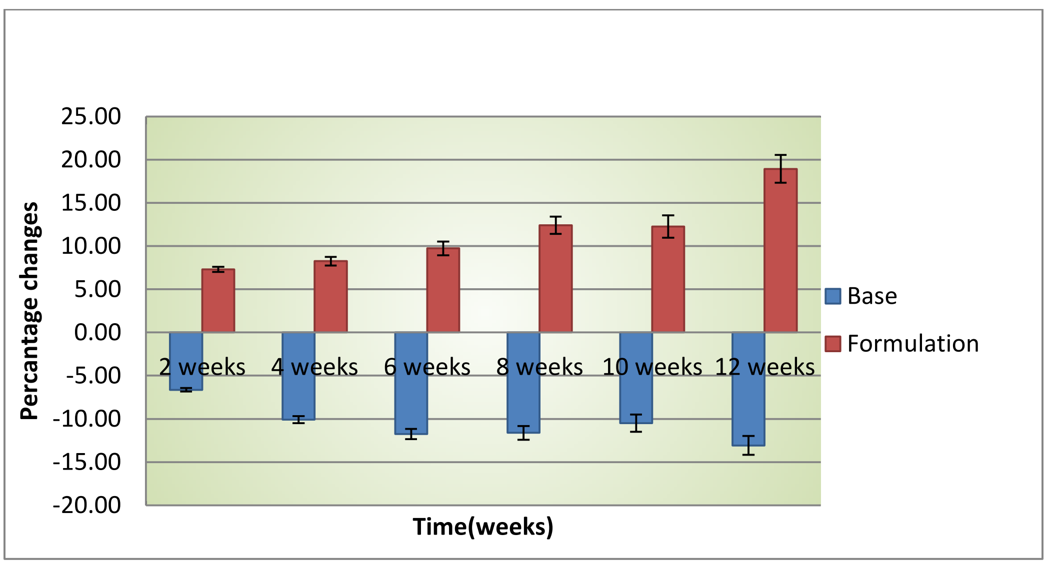


Figure 4.57. Percentage changes in skin sebum contents after application of GB and GF

4.1.6.2.7. Surface Evaluation of Living Skin (SELS)

Visioscan VC 98/software SELS 2000 (Courage and Khazaka GmbH,Germany) is used for the measurement of SELS parameters SE*w* , SE*sc*, SE*sm* and SE*r* before the application of emulsions containing grape seeds extract and then at 1st, 2nd and 3rd months of the study duration are given in **(Table. 4.43)**. An example of typical and 3D image taken by SELS 2000 software of a volunteer facial skin at base line and after 3 months of treatment has been shown in **(Figure. 4.58-4.61).**

Table 4.43. SELS parameters values(Mean±SD)

Parameters	Emulsion	0 hour	1month	2 month	3 month
SEr	Base	3.07±0.178	3.08±0.177	3.07±0.180	3.08±0.179
	Formulation	3.02±0.176	2.98±0.172	2.93±0.173	2.89±1.63
SEsc	Base	1.66±0.041	1.66±0.044	1.66±0.042	1.66±0.049
	Formulation	1.66±0.049	1.62±0.048	1.58±0.049	1.53±0.047
SEsm	Base	100.22±4.91	100.22±4.93	100.03±4.89	100.82± 4.88
	Formulation	100.22±4.90	97.28±5.02	94.95±4.92	92.03± 5.02
Sew	Base	60.51±2.77	60.50±2.78	60.52±2.76	60.49±2.77
	Formulation	59.45±2.73	59.39±2.74	58.20±2.72	57.16±1.63

SEr ;skin roughness, , SEw ;skin wrinkles, , SEsm; skin smoothness SEsc ;skin scaliness

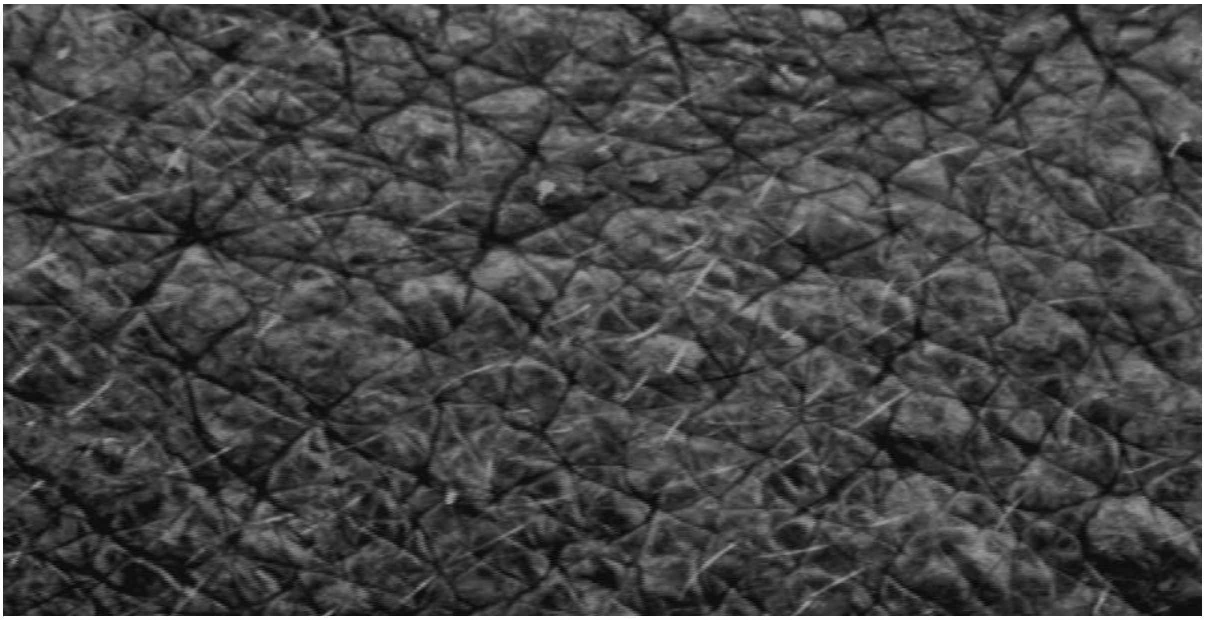


Figure 4.58. An image of skin taken by Visioscan® before application of GF

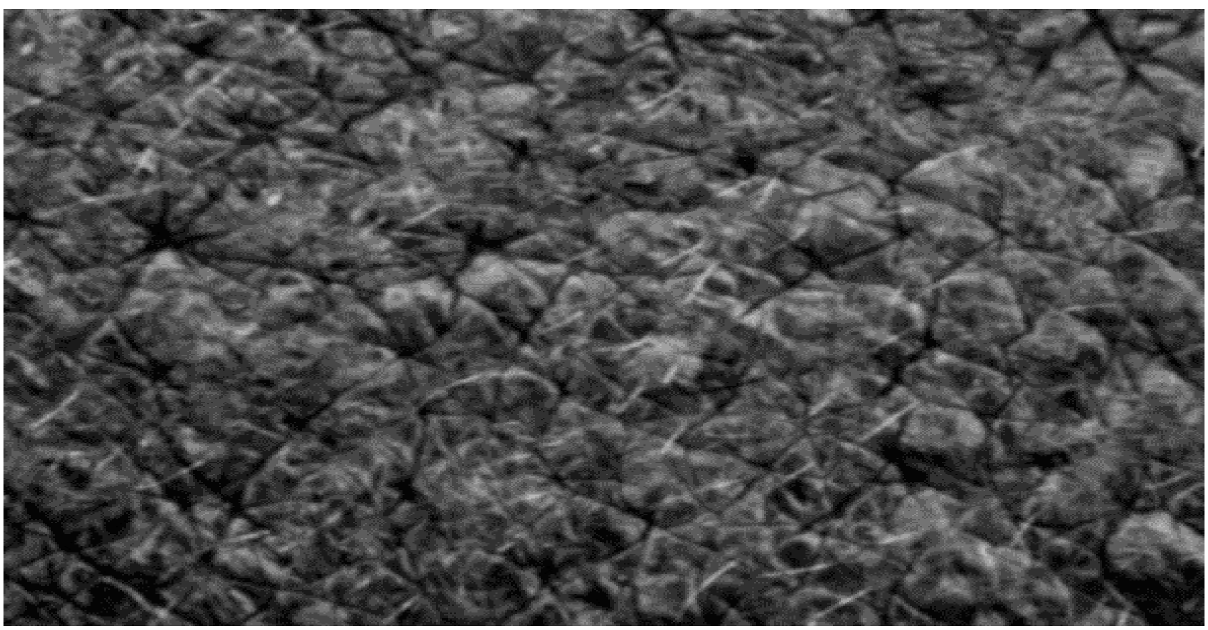


Figure 4.59. An image of skin taken by Visioscan® after 3 months of application of GF

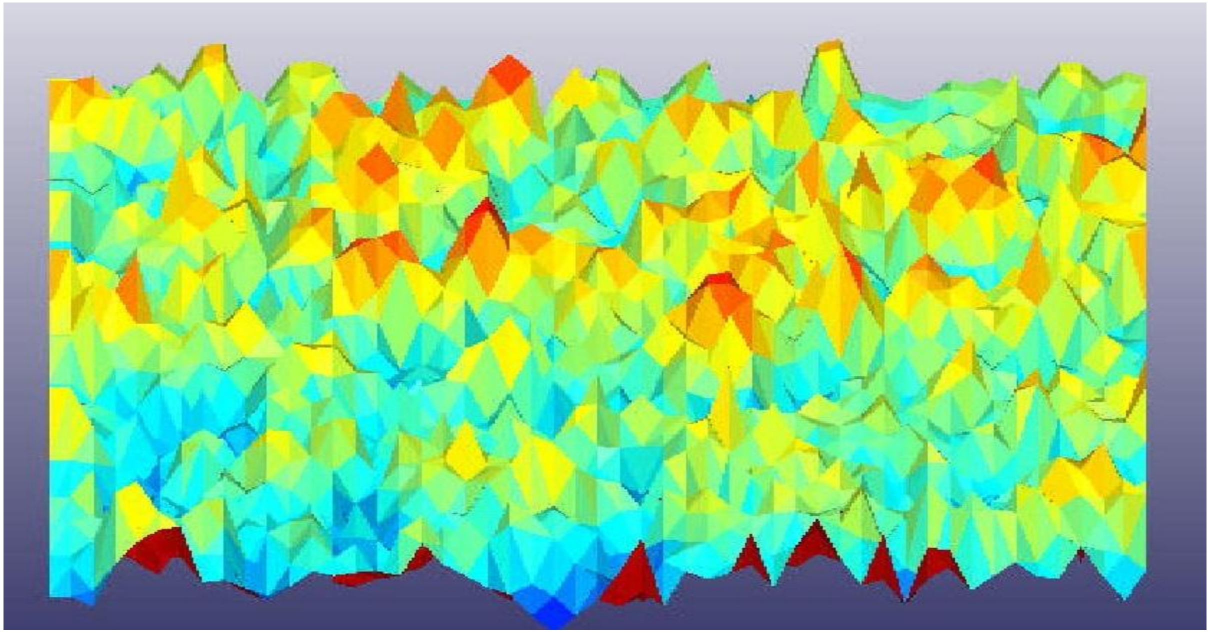


Figure 4.60. 3D image of skin taken by Visioscan® before application of GF

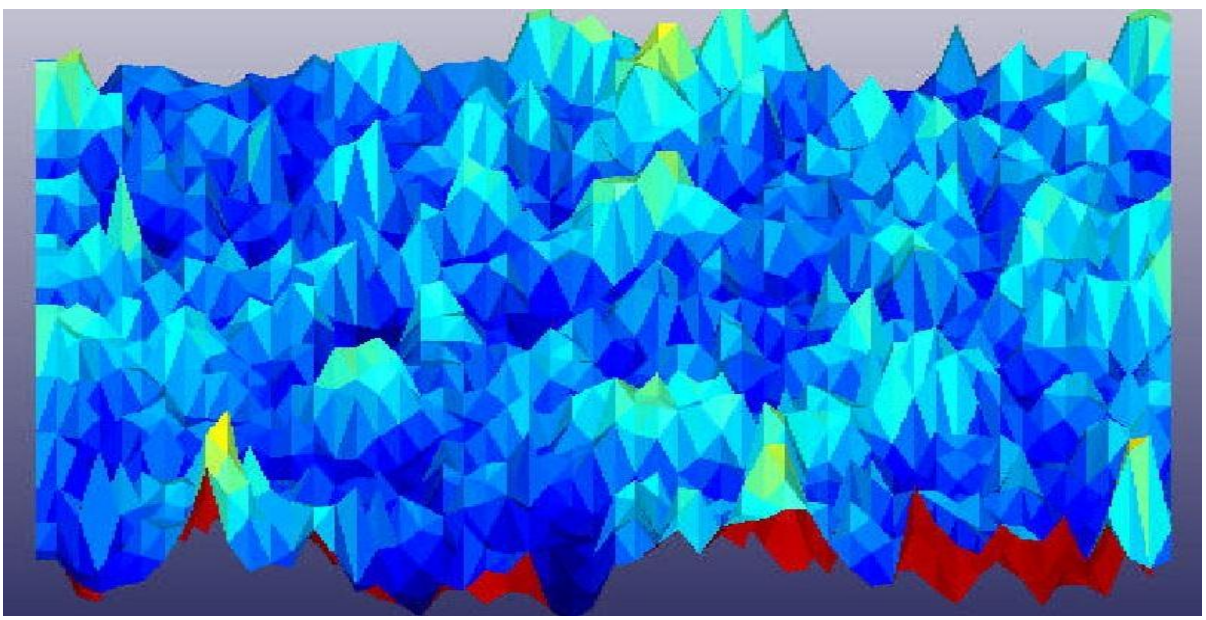


Figure 4.61. 3D image of skin taken by Visioscan® after 3 months of application of GF

4.1.6.3. Emulsion containing crude extract of Tamarind seeds

4.1.6.3.1. Skin sensitivity (Patch Test) for emulsion containing Tamarind seeds extract

Patch test for emulsion containing extract of tamarind seeds was performed with same procedure as adopted emulsions containing soybean seeds extract and grape seeds extract. Each score with respect to volunteers is given in **(Table. 4.44).**

Table 4.44. Score given by volunteers to TB (Tamarind base) and TF(Tamarind formulation) on the basis of itching and irritation

Score		0	1	2	3
No. volunteers	TB	8	2	2	0
	TF	9	2	1	0

4.1.6.3.2. Panel test

Same procedure for Panel test for emulsion containing tamarind seeds extract was performed as adopted with emulsion containg soybean and grape seeds extract . Paired sample t-test was applied and it was found that there was an insignificant difference between the average points of sensitivity for Base and Formulation. Both the emulsions have similar performance from the sensory point of view **(Table. 4.45).**

Table 4.45. Average Values ± SEM for Panel Test by 11 Volunteers for TB and TF

	Average points for Base ± SEM	Average points for Formulation ± SEM
Ease of application	4.12 ± 0.03	4.13± 0.10
Spreadability	4.20 ± 0.04	4.25± 0.05
Sense just after application	4.00± 0.06	4.00 ± 0.07
Sense in long term	4.16 ± 0.07	4.15± 0.08
Irritation	0.00 ± 0.00	0.00 ± 0.000
Shine on skin	4.11± 0.08	4.22± 0.03
Sense of softness	4.32 ± 0.05	4.37± 0.06

4.1.6.3.3. Melanin and erythema

The percent change with respect to time in melanin and erythema following the applications of the Tamarind base(TB) and Tamarind formulation(TF) on the cheeks of human volunteers has been presented in tables **(Table. 4.46-4.49) and** represented by figures **(Figue. 4.62-4.63)**.

Table 4.46. Percentage of change in skin melanin content after application of TB

Volunteer No.	2nd week	4th Week	6th Week	8th week	10th Week	12th Week
1	1.00	2.81	9.02	13.43	9.62	9.82
2	0.75	3.21	-1.51	3.21	3.40	7.17
3	6.94	1.57	9.62	4.70	-1.12	-2.24
4	1.62	-0.36	-1.62	-4.14	1.98	-7.01
5	-1.46	-6.65	-4.57	-2.70	-0.42	-0.83
6	1.35	2.13	3.09	2.51	1.35	-0.19
7	-0.79	-1.19	-2.77	1.78	7.31	3.75
8	2.98	5.17	4.17	7.16	9.54	13.12
9	2.41	3.41	2.21	-0.80	1.41	2.01
10	1.54	0.77	5.41	-0.39	5.02	6.76
11	3.85	0.68	4.98	-0.45	5.88	10.63
Mean ± SEM	**1.84±0.69**	**1.05±0.95**	**2.55±1.42**	**2.21±1.50**	**4.00±1.14**	**3.91±1.87**

Table 4.47. Percentage of change in skin melanin content after application of TF

Volunteer No.	2^{nd} week	4^{th} week	6^{th} week	8^{th} week	10^{th} Week	12^{th} Week
1	-0.66	2.19	-0.44	-1.97	-2.84	-5.25
2	-2.14	-4.93	-3.00	-3.85	-4.71	-5.78
3	-0.21	-3.59	-1.69	-0.42	-5.49	-8.86
4	-1.59	-0.88	-3.72	-2.65	-4.60	-7.08
5	0.38	1.34	-19.39	-1.15	-1.73	-2.88
6	-1.72	-2.58	-4.29	-5.15	-6.22	-6.87
7	2.90	1.55	3.48	0.39	5.80	-2.71
8	5.69	4.60	2.19	0.44	-3.28	-4.16
9	1.43	-0.72	-1.25	-2.68	-3.76	-5.72
10	2.20	0.60	0.20	-2.20	-2.40	-4.01
11	-0.46	-2.31	-1.15	0.92	-3.23	-4.62
Mean ± SEM	**0.53±1.71**	**-0.43±1.85**	**-2.64±1.82**	**-1.67±1.58**	**-2.95±1.97**	**-5.27±1.56**

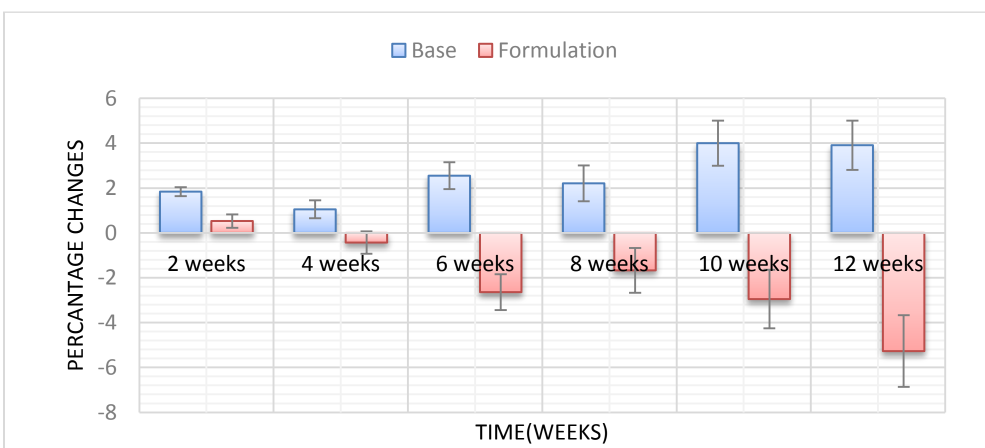


Figure 4.62. Percentage changes in skin melanin after application of TB and TF.

Table 4.48. Percentage of change in values of skin erythema after application of TB

Volunteer No.	2nd week	4th week	6th week	8th week	10th Week	12th Week
1	3.29	2.26	-4.12	-0.41	22.02	-1.23
2	-2.05	-1.03	-5.95	-2.87	-5.54	-5.34
3	1.71	3.23	3.80	2.28	0.95	0.38
4	0.46	4.10	2.28	0.68	4.10	-0.68
5	5.50	8.50	7.50	1.75	3.75	0.25
6	-0.63	0.95	2.22	2.85	5.38	0.95
7	-2.18	0.00	0.97	2.91	-1.94	-3.63
8	0.60	-2.41	-1.81	-5.42	0.30	-6.33
9	-11.57	-12.40	-12.19	-12.81	-14.26	-7.64
10	-1.80	-1.44	-3.60	-2.70	-3.42	-4.32
11	2.15	4.31	5.87	4.70	2.94	-0.59
Mean ± SEM	**-0.41±1.33**	**0.55±1.61**	**-0.46±1.74**	**-0.82±1.51**	**1.30±2.68**	**-2.56±0.91**

Table 4.49. Percentage of change in values of skin erythema after application of TF

Volunteer No.	2nd week	4th week	6th week	8th week	10th Week	12th Week
1	2.62	-1.43	0.48	-0.24	1.19	-2.14
2	-1.81	-2.49	-3.63	-4.31	-2.95	-7.71
3	1.34	-0.38	-0.19	-2.29	-3.82	-5.16
4	1.89	3.07	2.12	0.71	0.24	-0.94
5	-0.64	-3.00	-2.36	-5.36	-7.30	-4.94
6	1.91	9.55	2.87	3.82	-0.32	-0.96
7	-4.09	-2.95	-0.91	-4.09	0.68	-5.00
8	1.28	2.56	-3.83	-2.24	0.32	-3.83
9	1.45	-1.69	-0.72	0.48	-2.41	-3.61
10	-4.81	-1.43	-5.35	-5.88	-3.39	-0.71
11	1.39	3.78	2.58	0.60	-0.40	-2.19
Mean ± SEM	**0.05± 0.77**	**0.51± 1.17**	**-0.81± 0.83**	**-1.71± 0.92**	**-1.65± 0.78**	**-3.38± 0.67**

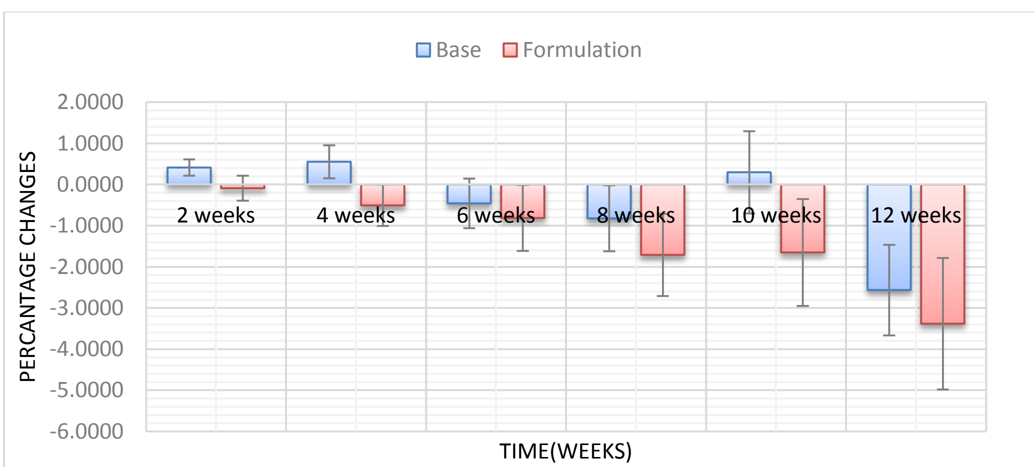


Figure 4.63. Percentage changes in skin erythema after application of TB and TF.

4.1.6.3.4. Skin moisture contents

The percentage of change in the measured skin moisture contents after applications of Tamarind base (TB) and Tamarind formulation(TF) have been given in tables**(Table. 4.50, 4.51)** and represented in figure **(Figure. 4.64).**

Table 4.50. Percentage of change in values of skin moisture contents after application of TB

Volunteer No.	2nd week	4th Week	6th week	8th week	10th Week	12th Week
1	-3.83	-0.55	1.64	2.46	-1.09	-2.19
2	-1.27	-3.17	3.17	6.03	7.62	8.57
3	1.96	10.13	3.27	10.46	13.07	14.05
4	-3.98	-6.63	-2.65	0.27	1.33	1.86
5	-3.83	2.68	-14.56	4.60	6.13	3.83
6	8.42	-21.61	-2.20	0.37	-15.38	0.00
7	-0.88	-7.96	3.54	5.60	-4.13	4.72
8	-1.02	6.44	7.80	9.83	10.51	0.00
9	3.85	0.89	4.14	-9.76	-1.18	2.37
10	-4.85	5.08	3.46	6.70	3.23	6.00
11	-3.79	-0.81	1.36	1.63	4.07	-4.61
Mean ± SEM	**-0.84± 1.24**	**-1.41± 2.60**	**0.82± 1.7**	**3.47± 1.69**	**2.20± 2.36**	**3.15± 1.57**

Table 4.51. Percentage of change in values of skin moisture contents after application of TF

Volunteer No.	2^{nd} week	4^{th} Week	6^{th} Week	8^{th} week	10^{th} Week	12^{th} Week
1	6.43	0.54	1.61	8.58	16.62	10.46
2	3.28	8.96	11.64	19.70	25.97	21.49
3	8.92	7.01	10.51	21.66	25.16	28.66
4	2.20	8.52	5.49	3.85	4.67	9.34
5	-3.66	17.95	26.01	30.04	38.46	32.60
6	3.39	-7.12	5.76	11.19	15.93	20.68
7	-6.21	15.22	3.11	4.66	9.01	20.19
8	4.29	12.54	18.15	20.46	23.10	27.72
9	3.38	8.17	3.10	18.59	5.35	10.99
10	13.18	25.72	-2.57	13.50	0.96	7.07
11	-2.17	9.88	20.00	14.70	21.45	17.35
Mean ± SEM	**3.00± 1.68**	**9.76± 2.60**	**9.35± 2.67**	**15.17± 2.39**	**16.97± 2.41**	**18.78± 2.60**

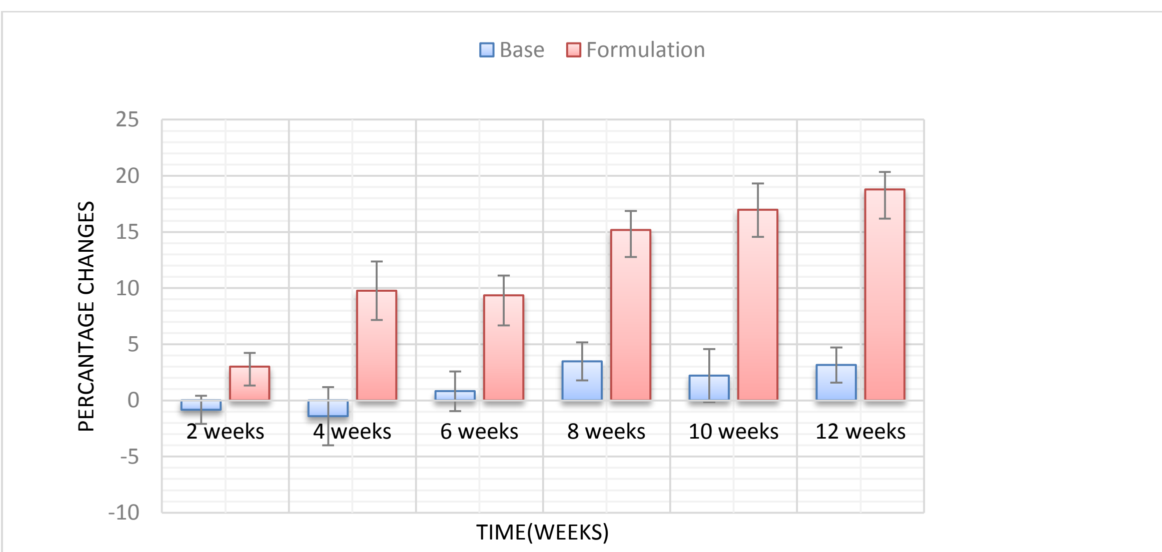


Figure 4.64. Percentage changes in skin moisture contents after application of TB and TF

4.1.6.3.5. Skin sebum contents

The percentage of change in the measured skin sebum values after applications of Tamarind base (TB) and Tamarind formulation(TF) have been given in tables **(Table. 4.52, 4.53)** and represented by figure **(Figure. 4.65)**.

Table 4.52. Percentage of change in values of skin sebum after application of TB

Volunteer No.	2^{nd} Week	4^{th} week	6^{th} week	8^{th} week	10^{th} Week	12^{th} Week
1	4.76	16.67	9.52	7.14	28.57	7.14
2	-3.33	-2.22	1.11	4.44	10.00	-3.33
3	-4.63	-7.41	-4.63	-12.04	-12.96	-13.89
4	5.08	8.47	10.17	-1.69	30.51	5.08
5	3.08	7.69	4.62	1.54	15.38	9.23
6	1.85	22.22	14.81	12.96	29.63	16.67
7	8.82	14.71	22.06	1.47	13.24	14.71
8	19.40	13.43	17.91	32.84	26.87	19.40
9	10.17	27.12	23.73	38.98	10.17	15.25
10	4.84	6.45	12.90	19.35	6.45	16.13
11	5.33	16.00	20.00	21.33	24.00	28.00
Mean ± SEM	**5.03± 1.97**	**11.19± 2.04**	**12.02± 2.71**	**11.48± 2.65**	**16.53± 2.99**	**10.40± 2.48**

Table: 4.53. Percentage of change in values of skin sebum after application of TF

Volunteer No.	2nd Week	4th Week	6th week	8th week	10th Week	12th Week
1	5.04	-2.52	-9.59	-17.16	-16.66	-18.19
2	-5.73	-8.1	-12.24	-18.75	-15.79	-17.57
3	3.86	-0.58	-7.29	-13.43	-14.71	-13
4	-7.4	-4.2	3.4	-2.6	-8.2	-12.2
5	2.94	-6.81	-13.9	-17.77	-14.55	-19.06
6	-4.88	-8.75	-27.36	-21.93	-25.03	-26.58
7	-7.82	-5.55	-15.77	-12.36	-19.18	-21.45
8	-5.32	-2.85	-12.11	-19.52	-22.6	-18.75
9	-5.76	-8.52	-17.67	-22.43	-16.48	-18.05
10	6.36	-2.79	-8.82	-16.18	-18.64	-17.07
11	-6.21	-9.33	-17.67	-13.5	-17.67	-15.58
Mean ± SEM	**-2.27± 1.67**	**-5.45± 0.92**	**-12.64± 2.32**	**-15.97± 1.67**	**-17.23± 1.33**	**-17.95± 1.18**

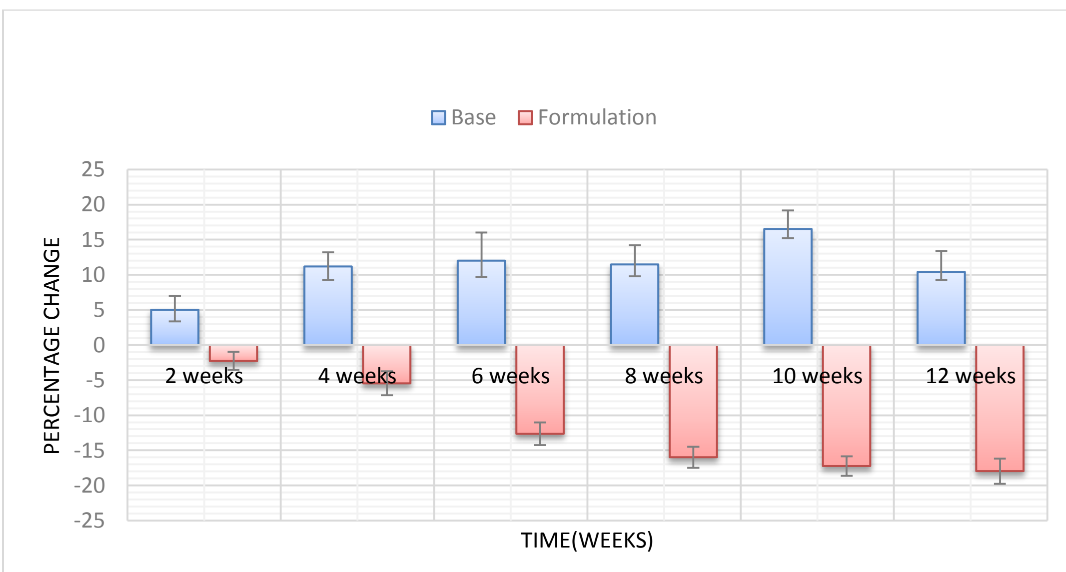


Figure 4.65. Percentage changes in skin sebum after application of TB and TF.

4.1.6.2.6.Skin Elasticity

After 12 weeks study the percent changes in the values of skin elasticity following applications of Tamarind base (TB) and Tamarind formulation (TF) have been given in tables **(Table. 4.54, 4.55)** and represented by figure **(Figure. 4.66).**

Table 4.54. Percentage of change in values of Skin Elasticity after application of TB

Volunteer No.	2nd Week	4th Week	6th Week	8th Week	10th Week	12th Week
1	-3.08	4.62	-6.15	-1.54	7.69	1.54
2	6.78	3.39	-10.17	6.78	-1.69	-1.69
3	1.47	-1.47	-1.47	-13.24	-8.82	4.41
4	6.15	-15.38	10.77	4.62	-10.77	6.15
5	2.94	11.76	5.88	-1.47	8.82	10.29
6	-1.27	-8.86	0.00	5.06	-6.33	-10.13
7	14.04	8.77	3.51	22.81	1.75	10.53
8	1.43	4.29	1.43	-8.57	7.14	1.43
9	-3.08	4.62	12.31	6.15	16.92	-1.54
10	-6.06	7.58	-3.03	-4.55	1.52	4.55
11	7.35	-1.47	10.29	2.94	5.88	0.00
Mean ± SEM	**2.43± 1.76**	**1.62± 2.41**	**2.12± 2.19**	**1.73± 2.87**	**2.01± 2.54**	**2.32± 1.78**

Table 4.55. Percentage of change in values of Skin Elasticity after application of TF

Volunteer No.	2nd Week	4th Week	6th Week	8th Week	10th Week	12th Week
1	21.82	12.73	20.00	23.64	30.91	41.82
2	-11.67	13.33	-6.67	8.33	13.33	25.00
3	6.35	4.76	7.94	15.87	28.57	23.81
4	-8.82	-2.94	2.94	0.00	20.59	16.18
5	7.69	23.08	27.69	0.00	21.54	13.85
6	31.15	52.46	42.62	42.62	31.15	19.67
7	-12.16	10.81	-5.41	14.86	12.16	22.97
8	8.57	1.43	-2.86	-1.43	11.43	10.00
9	4.17	1.39	6.94	5.56	-12.50	1.39
10	8.33	18.33	26.67	18.33	13.33	20.00
11	1.27	-5.06	-1.27	-5.06	-12.66	7.59
Mean ± SEM	**5.15±2.03**	**11.85±2.87**	**10.78±1.99**	**11.16±2.21**	**14.35±1.60**	**18.39±2.24**

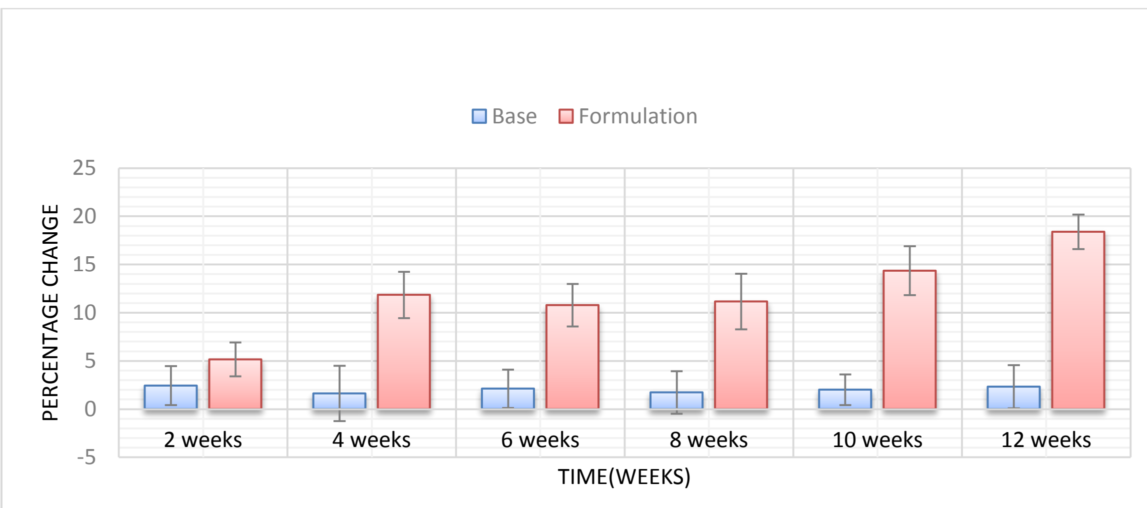


Figure 4.66. Percentage changes in skin elasticity after application of TB and TF.

4.1.6.2.7. Surface Evaluation of Living Skin (SELS)

Visioscan VC 98/software SELS 2000 (Courage and Khazaka GmbH) is used for the measurement of SELS parameters SE*w* , SE*sc*, SE*sm* and SE*r* before the application of emulsions containing tamarind seeds extract and then at 1st, 2nd and 3rd months of the study duration are given in **(Table. 4.56)**. An example of typical and 3D image taken by SELS 2000 software of a volunteer facial skin at base line and after 3 months treatment has been shown in **(Figure. 4.67-4.70)**

Table 4.56. SELS parameters values(Mean±SD)

Parameters	Emulsion	0 hour	1month	2 month	3 month
SEr	**Base**	4.02±0.054	4.05±0.09	4.01±0.080	4.06±0.075
	Formulation	4.03±0.082	3.98±0.063	3.92±0.073	3.82±0.081
SEsc	**Base**	1.73±0.040	1.73±0.048	1.72±0.053	1.72±0.056
	Formulation	1.71±0.038	1.68±0.051	1.63±0.053	1.60±0.045
SEsm	**Base**	107.99±4.81	109.98±4.80	108.64±3.78	107.82± 2.82
	Formulation	101.01±4.65	97.51±4.80	95.12±2.33	89.02± 2.12
Sew	**Base**	70.63±1.42	71.72±1.34	70.84±1.34	71.80±1.00
	Formulation	65.73±1.42	63.73±1.33	61.82±1.35	58.31±1.34

SEr ;skin roughness, , SEw ;skin wrinkles, , SEsm; skin smoothness SEsc ;skin scaliness

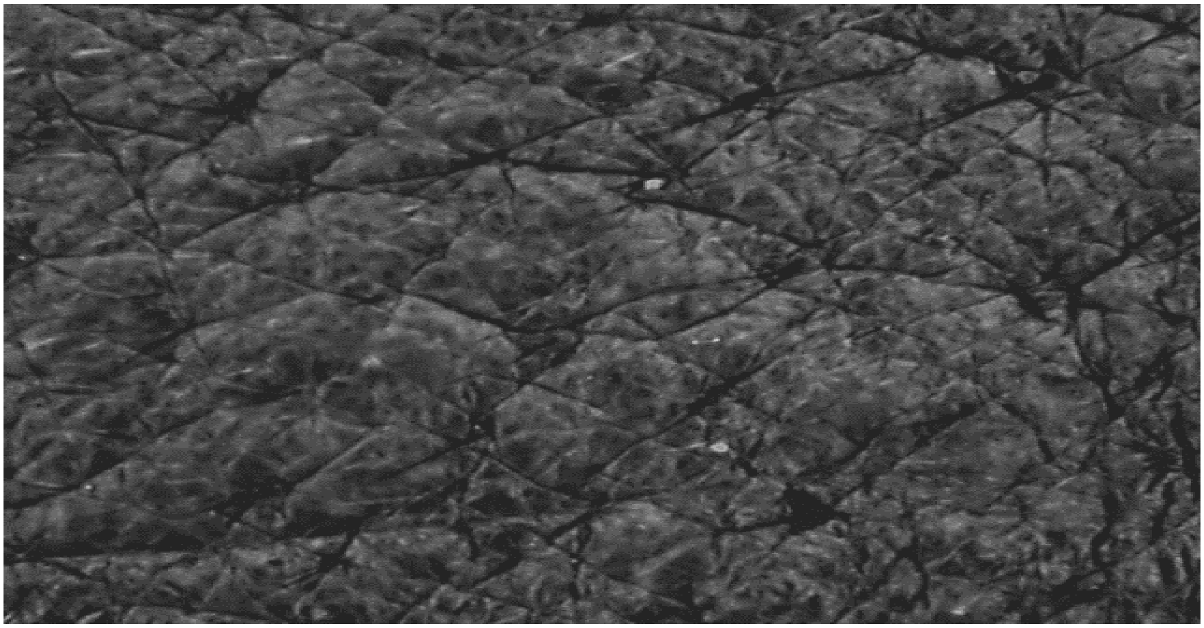


Figure 4.67. An image of skin taken by Visioscan® before application of TF

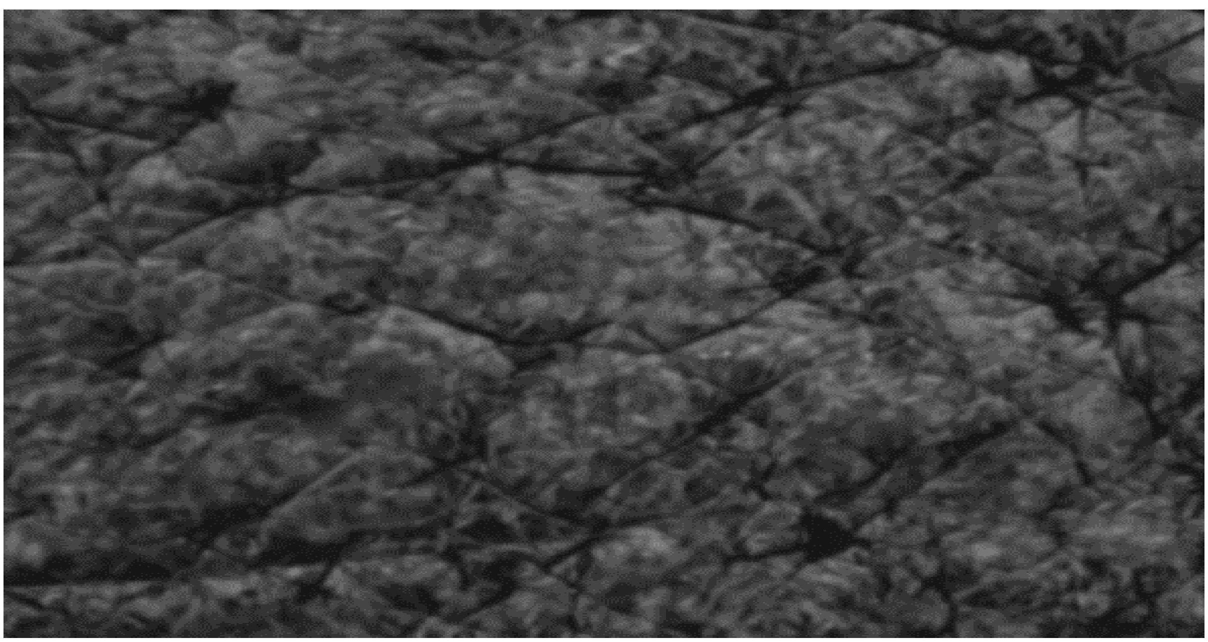


Figure 4.68. An image of skin taken by Visioscan® after 3 month application of TF

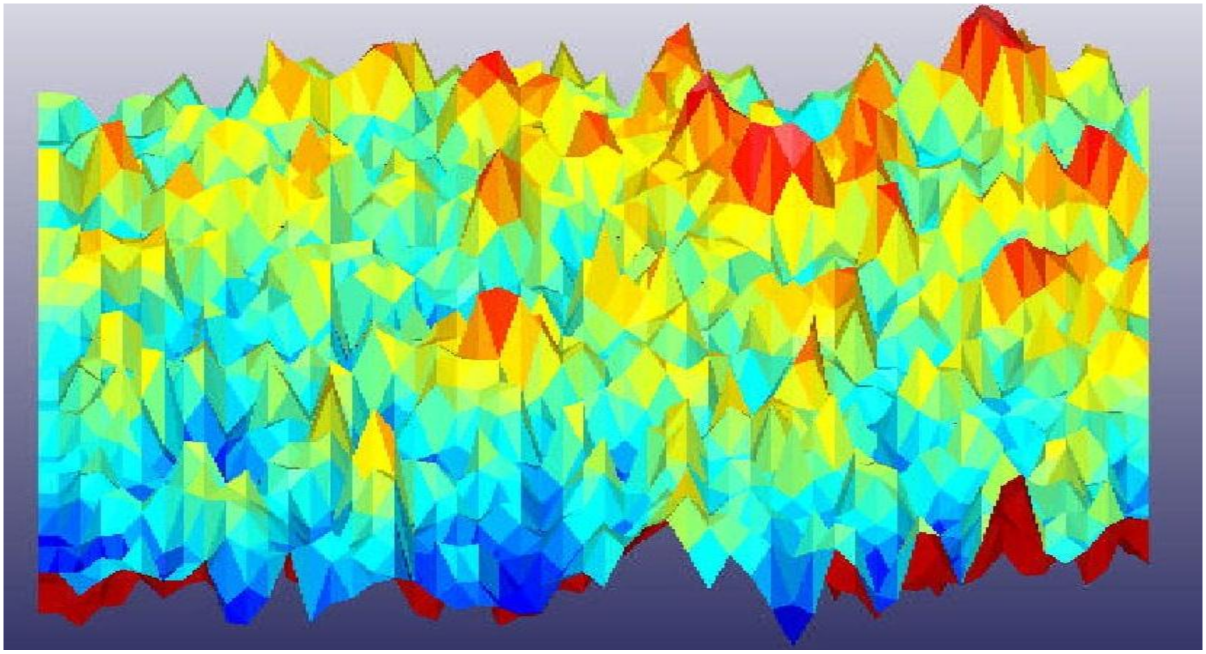


Figure 4.69. 3D image of skin taken by Visioscan® before application of TF

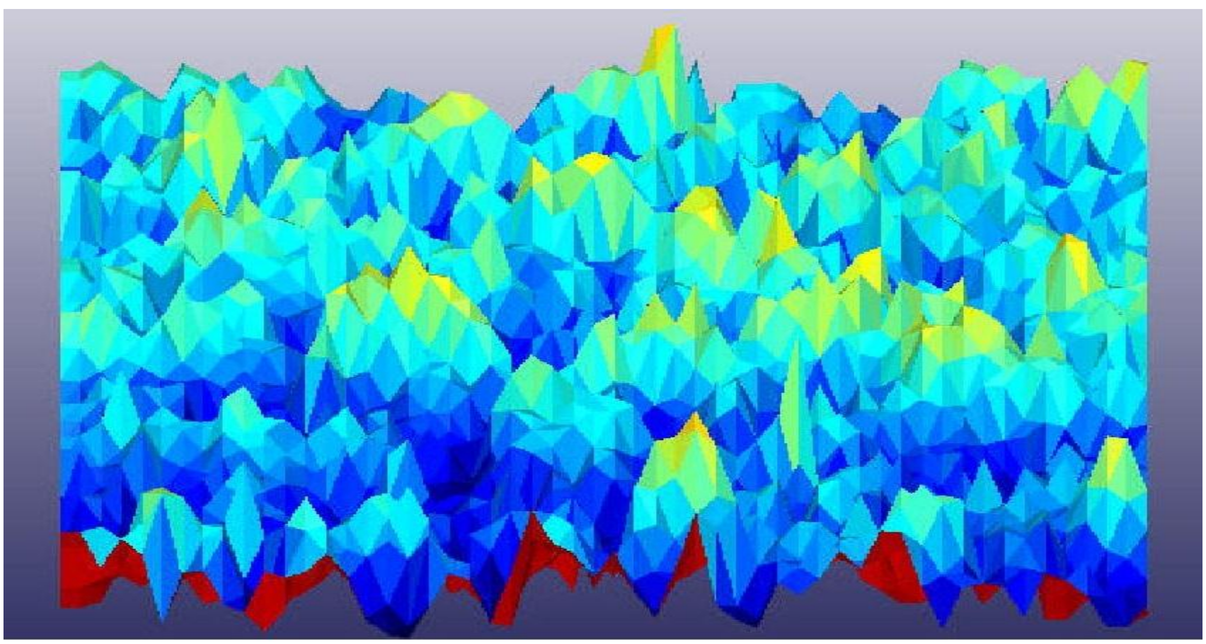


Figure 4.70. 3D image of skin taken by Visioscan® after 3 months of application of TF

4.2 Discussion

4.2.1. Antioxidant activity of botanical extracts

The antioxidant activity of plants is mainly contributed by the active compounds. Various plants have different free radical antioxidant activity which depends upon their different constituents.

The 50% ethyl alcoholic extract of *Vitis vinifera* seeds showed 85.61% DPPH scavenging activity when ascorbic acid was used as a standard. The antioxidant activity of grape seed extract, primarily resides in the potent antioxidant proanthocyanidins. These polyphenolic bioflavonoids are also known as procyanidins, procyandiol oligomers, leukoanthocyanidins, condensed tannins, and oligomeric proanthrocyanidins (OPCs), and they comprise 60% of poly phenols (Chaturvedi et al., 2011). The OPCs consist of dimers of catechins and oligomers of epicatechin and catechin and their gallic acid esters. These compounds are scavengers of both reactive oxygen and nitrogen species Grape seed also includes other therapeutic compounds including flavonoids such as kaempferol and quercetin glucosides; stilbenes such as resveratrol and viniferins; fruit acids; tocopherols; essential fatty acids; and phenylacrylic acids such as caffeoyl and feruloylsuccinic acid. These compounds are potent antioxidants (Fu et al., 2007). Extracts of soybeans and tamarind showed 83.45% and 79.2% free radical scavenging activity, respectively which were significantly ($P<0.05$) different from each other as well as significantly ($P<0.05$) different from that of *Vitis vinifera*. The present study confirms 85.61% antioxidant activity of grape seeds extract when 50% ethanol is used as solvent. It indicates that ethanol water mixture gives better extraction of more total flavonol compounds and procyanidins which are potent antioxidants. The presence of water increases the permeability of seed tissue and thus enables a better mass transport by molecular diffusion (Waqas et al., 2013). Several bioactive compounds, including phenolics such as phenolic acids, flavonoids and isoflavonoids have been identified in soybeans. Recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activity of these phenolic compounds (Kim et al., 2006). Genistein, the major component of soybean isoflavones, has been demonstrated to inhibit ultraviolet -B (UVB)-induced skin tumorigenesis in hairless mice. The antioxidant properties of genistein may explain the mechanisms of its anti photocarcinogenic action because through either direct quenching of reactive oxygen species or indirect anti-inflammatory effects, genistein was found to substantially inhibit a series of oxidative events elicited by UVB irradiation, including hydrogen peroxide production, lipid peroxidation, and 8-hydroxy-2.- deoxyguanosine formation (Lee et al., 2005) . The present study investigated 83.45% DPPH activity of soybean seeds extract when extraction was done with ethanol: hexane 1:1 solvent mixture. This solvent mixture gives a better extraction of soy isoflavones. *Tamarindus indica* L. seeds are important sources of antioxidant activity as 2-hydroxy-3',4'-dihydroxyacetophenone, methyl 3,4- dihydroxybenzoate, 3,4-dihydroxyphenylacetate and (-)-epicatechin, in addition to oligomeric proanthocyanidins (OPCs) . In the present study, antioxidant activity (i.e. 79.26%) is evaluated when hexane-ethanol-acetone (50:25:25) mixture is used for extraction of *Tamarindus indica* L. seeds. This solvent mixture gives the more efficient extraction of oligomeric proanthocyanidins (OPCs), catechin and epicatechin type compounds from *Tamarindus indica* seeds, which are important and the most plentiful antioxidants (Luzia and Jorge, 2011).

4.2.2. Stability testing

Stability of emulsions containing botanical extracts of soybeans, grape seeds and tamarind was evaluated. Stability testing is an integral part for emulsion development work. The formulator during the manufacturing of emulsion is concerned with the understanding the effects of storage and shipping conditions on shelf life which may include extremes of temperatures and exposure to sunlight, vibration and humidity. Formulations hence stored under different conditions and carefully examined at period intervals. Break down of emulsions will be strongly influenced by environmental conditions (temperature, humidity), pH, composition factors and processing conditions. The accelerated conditions like temperature may successfully be applied to W/O emulsions for their Changes in temperature significantly changes interfacial tension, viscosity, surfactant’s nature, thermal agitation of molecules and vapor pressure of liquid phase (Muhammad et al., 2014). It represents that emulsions tend to be very sensitive to temperature. It is observed that emulsion stability decreases with rise in temperature. Each emulsion i.e., formulation and base were divided in to four samples separately and these samples were kept at different storage conditions i.e. at 8±0.5°C(Refrigerator), 25±0.5°C, 40±0.5°C, 40±0.5°C + 75% RH (Relative Humidity) in incubators(Waqas et al., 2010). All the three emulsions containing botanical extracts of soybean, grape seeds and tamarind along with their respective bases were examined organoleptically with respect to change in color, liquefaction and phase separation for a period of 90 days at definite time intervals.

4.2.2.1 Color

The color of freshly prepared soybean base and soybean formulation is white and pale yellow respectively. Up to observation period of three months there is no change in color of both base and formulation. This showed that the emulsions were stable at different storage conditions i.e. 8^0C± 0.5∘C, 25^0C± 0.5°C, 40^0C± 0.5°C and 40^0C± 0.5°C + 75% RH throughout three months study period **(Table. 4.1)**. No change in the color may be attributed to different factors contributing the emulsion stability. As the soy bean seeds extract contains polyphenols. Polyphenols have inhibitory activity against bacterial growth. The mechanism of polyphenol toxicity against microbes may be the inhibition of hydrolytic enzymes. This may play a role in protecting the formulation from degradation by those organisms that produce such substances which bring about changes in the color of the formulation during the storage (Akhtar et al., 2010c).

Physical characteristics and visualization regarding the stability of the entire four samples kept at 8± 0.5°C, 25± 0.5°C, 40± 0.5°C and 40± 0.5°C +75% RH containing grape seeds extract were studied and presented in (**Table. 4.2)** . The freshly prepared emulsion samples were light pinkish in color. The light pinkish color of samples was due to the presence of grape seed extract. There was no change in the color of the emulsion samples up to the observation period of 90 days. This represents that emulsion formulation was stable at different storage conditions i.e. 8^0C, 25^0C, 40^0C and 40^0C + 75% RH throughout the period of observation, i.e. 90 days. The stability of the formulations throughout the observation period may be attributed to different contributing factors. Such as the ingredients of non-aqueous portion like paraffin oil, which is mixture of hydrocarbons and a transparent, non-fluorescent liquid(Liu et al., 2006) and Abil-EM90 which is a clear, colorless and nontoxic liquid emulsifying agent(Choi et al., 2009). Grapes are rich in polyphenols and 60–70% of grape polyphenols are found in grape seeds. The grape seed polyphenols are flavan-3-ol derivatives. The major compounds are catechins, epicatechin, epicatechin-3-O-gallate, procyanidins dimers (B1-B5), procyanidin C1, and procyanidin B5-3′-gallate(Shi et al., 2003). Polyphenols contain established antimicrobial activity against a vast majority of bacteria such as *Shigella flexneri ,Staphyllococcus aureus.* Oxidized polyphenols also have inhibitory activity against bacterial growth. The mechanism of polyphenol toxicity against microbes may be the inhibition of hydrolytic enzymes (Karou et al., 2005). This may play a role in protecting the formulation from degradation by those organisms that produce such substances which bring about changes in the color of the formulation during the storage.

In case of emulsion containing tamarind seeds extract, the freshly prepared base was white and formulation was pinkish in color. The pinkish color of formulation was due to the presence of *Tamarindus indica* seeds extract which contains carotenoids (De Caluwé et al., 2010). There was no change in the color of both the base and the formulation up to the observation period of 90 days. This showed that both of the formulations were stable at different storage conditions *i.e.* 8 ±0.5°C, 25±0.5 °C, 40±0.5 °C, and 40±0.5 °C + 75% RH throughout the period of analysis, i.e. 90 days **(Table. 4.3)**. It has been reported the *Tamarindus indica* seeds extract presented antimicrobial activity against the selected organism due to the phenolic compounds and flavonoids. The most sensitive microorganisms were *Escherichia coli* and *Staphylococcus aureus* (Ghelardi et al., 2004). From the previous studies it was proved that the microbial growth in formulations may destroy the constituents which are capable to change formulation color. But the polyphenols contents prevent microbial growth.

4.2.2.2. Liquefaction

The effective viscosity in water-in-oil emulsion (W/O) is mainly dependent on volume fraction of dispersed phase and temperature along with minor factor such as shear rate, average droplet size, droplet size distribution and density of the oil used in formulations (Akhtar et al., 2011).

During the study period of emulsion containing soybean seeds extract, no liquefaction was observed in any of the samples of base and formulation kept at 8±0.5°C and 25±0.5°C but the slight liquefaction was observed in base samples kept at 40±0.5°C and 40±0.5°C &75% RH on 90^{th} day**(Table. 4.1)**.

All samples of emulsion formulation containing grape seeds extract kept at $8±0.5^0$C and $25±0.5^0$C were stable for the period of 90 days and no liquefaction was seen in them. Very little liquefaction was seen in the sample of base kept at $40±0.5^0$C on 90^{th} day. Likewise a little liquefaction was also seen in formulation samples kept at $40±0.5^0$C on 90^{th} day of observation period**(Table. 4.2)**.

In case of base and formulation containing extract of tamarind seeds, no liquefaction was observed in any of the samples of base and formulation kept at 8±0.5°C and 25±0.5°C. Slight liquefaction was observed in samples of both base and formulation kept at $40±0.5^0$C on 90^{th} day of observation period**(Table. 4.3)**. The viscosity of emulsions is a key factor in their flow characteristics. Viscosity is a very practicable tool to check the quality and stability of the emulsion. As time elapses the prepared emulsions are prone to temperature and time dependent degradative processes which leads to decrease in viscosity and increase in liquefaction. In W/O emulsion, the creaming takes place due to the sedimentation of water droplets and forms the lower layer. According to the Stokes law, the rate of sedimentation is inversely proportional to the viscosity of the continuous phase. Due to this factor on increasing creaming, the viscosity of the formulation and the base gradually decreases at increased temperature which leads to liquefaction (Muhammad et al., 2014).

4.2.2.3. Phase separation of emulsions

Creaming (or settling) is due to differences in density between the two phases under the influence of gravity which leads to phase separation. The separated phase can either cream or sediment. Creaming is the upward movement of dispersed droplets relative to the continuous phase, while sedimentation, the reverse process, is the downward movement of particles. The emulsions are not thermodynamically stable and droplets (water) merge with each other to produce big droplets and increase the coalescence rate. Coalescence is one of the possible mechanisms of destruction of emulsions, which occur when the energy of adhesion between two droplets is larger than the turbulent energy causing dispersion (Robins, 2000).

During the study duration of the emulsion containing soybean seeds extract, the samples of base which were kept at 8 ± 0.5^0C and 25 ± 0.5^0C show stability but slight separation was shown by the samples those were kept at 40^0C and 40^0C+ 75% RH on 90^{th} day of observation, while in case of samples of formulation, there was no phase separation in any of the samples kept at 8 ± 0.5^0C, 25 ± 0.5^0C, 40 ± 0.5^0C and 40 ± 0.5^0C+ 75% RH up to observation period of 90 days (**Table. 4.1)** .

In case of emulsion containing grape seeds extract, there was no phase separation in any of the samples of emulsion formulation kept at 8 ± 0.5^0C, 25 ± 0.5^0C, 40 ± 0.5^0C and 40 ± 0.5^0C+ 75% RH up to observation period of 90 days(**Table. 4.2)**. Even at higher temperature no oily phase separation was monitored during the entire study duration.

The emulsion containing tamarind seeds extract shows no phase separation at 8 ± 0.5^0C and 25 ± 0.5^0C. but slight separation was shown by the samples those were kept at 40 ± 0.5^0C and 40 ± 0.5^0C+ 75% RH on 90^{th} day of observation, while in case of samples of formulation, there was no phase separation in any of the samples kept at 8 ± 0.5^0C, 25 ± 0.5^0C, 40 ± 0.5^0C and 40 ± 0.5^0C+ 75% RH up to observation period of 90 days(**Table. 4.3)**.

A number of stability factors like availing ABIL EM 90 which is a heat stable emulsifying agent. If phase separation is considered as a parameter of stability the formulation shows better stability than the base at higher temperatures. In the case of base, little phase separation at higher temperatures may be due to the movement of small number of molecules of the emulsifying agent from interface to the surface. This phenomenon is much easier at the low viscosity of the emulsion. In some conditions emulsions show higher stability at low temperatures because of increased phase viscosity (Nour and Yunus, 2006).

4.2.2.4. Centrifugation test

The process of centrifugation is based on the principle of separating the two or more substances having different densities using the centrifugal force, for example two liquids or a liquid and a solid. This is a very useful tool for evaluation and prediction of the shelf life of emulsions (HERBERT A. L., 2005). In the present study centrifugation test was employed to check the phase separation in the samples of base and the formulation kept at different temperatures up to a period of 90 days at definite time intervals. On centrifugation, there was no phase separation observed in any of the samples kept at different storage conditions i.e.

8±0.5^{0}C, 25^{0}C, 40±0.5^{0}C and 40±0.5^{0}C+ 75% RH up to 90th day of observation. This showed that the emulsions were stable at all the storage conditions for 90 days.

During emulsion formulation, the proper homogenization speed is very critical as it prevents the base and the formulation breakage during stress testing (Floury et al., 2000).

4.2.3. Rheological studies of emulsions

The flow characteristics of a cosmetic emulsion are clearly among some of its more important physical features in either technical or aesthetic terms. Hence the ability to measure, adjust and, if possible, forecast such characteristics is very important (İbanoğlu, 2002).

The shear stress causes strain in solids and liquids; the solid deforms and liquid flows. Rearrangement takes place inside the material due to stress application. In a purely viscous material, all the energy required to produce the deformation is dissipated as heat. On the contrary, in a purely elastic material, all the energy required to produce the deformation is stored. Dispersed phase affects the rheology of the emulsion by its globule size, volume concentration viscosity and chemical constituents. Rheological analysis allows the characterization of emulsions, to follow changes in emulsion induced by aging, shear and temperature and to predict their stability (Binks et al., 2005).

Rheological parameters of formulated emulsions were performed at 25±0.5ºC. Changes in viscosity were noted when shear stresses were applied. Rheogram of shear stress versus shear rate were obtained for all the formulations (Jiang et al., 2011). Power Model (PL) math model provided a numerically and graphically analyze the behavior of data sets. Power law model is a useful rheological model that describes the relationship between viscosity or shear stress and shear rate over the range of shear rates where shear thinning occurs in Non-Newtonian fluid (Ali et al., 2012a).

A computerized cone plate Rheometer was used in this study. Rheological parameters were performed at 25±0.5ºC . Changes in viscosity were recorded by applying shear stress. Rheological tests were performed on freshly prepared base and samples of all emulsions containing different botanical extracts stored at different temperatures. Rheogram of shear stress versus shear rate were obtained for all the emulsions. Viscosities were found to decrease in parallel to increase in shear stress. Viscosities were also found to decrease in formulations kept at different storage conditions especially at 40±0.5°C.

It was observed from the different study that when temperature was increased, the flow of molecules through interface is also increased. The flow of molecule correlated with viscosities. The viscosity is very sensitive to the temperature hence; the increment in temperature caused reduction of emulsion viscosity(Naveed, 2001). The Flow index values (n) for base and formulation were given in tables **(Table. 4.4-4.8, 4.9-4.13 and 4.14-4.18).**
The Flow index values indicated that cosmetic emulsions containing botanical extracts act as a non-newtonian fluid (Pseudoplastic fluid). The result were in agreement with saravacos, kostoropolous and Pal (1995) that emulsions show a marked non-newtonian behavior and most fruit and vegetable fluids and pastes are pseudoplastic, where the flow behavior index varies between 0 to 1 (Saravacos and Kostaropoulos, 1995). Power Law (PL) was found to fit to all the Rheogram and confidence of fit was found to be in the range of 97 to 99 %.The rheograms of all emulsions showed non-newtonian behavior, with flow index less than 1 which was an agreeable rheological property reflecting their pseudoplastic tendency. Emulsions with pseudoplastic flow properties cause the formation of a coherent film covering the skin surface. This characteristic is valuable and critical for a better phenolic antioxidant fortification of the skin surface (Ali et al., 2012a).

4.2.4. Skin evaluation parameters

4.2.4.1. Skin Melanin Contents

The overproduction of epidermal melanin, which is a high molecular weight, dark-brown colored compound, causes hyperpigmentation of the human skin(Slominski et al., 2004). Hyperpigmentation is a common skin problem that is particularly prevalent in middle aged and elderly individuals. It is cosmetically important and can greatly detract from both appearance and quality of life, particularly in cultures where smooth skin is valued as sign of health or in cultures that are beauty conscious (Briganti et al., 2003). Melanin is synthesized by melanosome in melanocyte. There are several functions of melanin in human body such as the synthesis of vitamin, prohibition of photocarcinogenesis and defense of skin from UV radiations. Skin color is determined by various substances like carotenoids, hemoglobin and melanin. The most important substance among them is melanin. Melanosomes are specialized lysosomal related organelles which are produced by the melanocytes; the pigment producing cells of follicular and interfollicular epidermis. The synthesis of biopolymers of melanin occurs within melanosomes (Slominski et al., 2004).

The effect of the base and formulation containing soybean seeds extract on skin melanin was examined with the help of Mexameter MPA 5(Courage and Khazaka GmbH, Germany). In this research, the base improves the skin melanin contents **(Table. 4.20)** but in case of formulation there is regular decline in skin melanin values throughout the study duration **(Table. 4.21 and Figure. 4.44)**. With the help of two-way ANOVA test defining a 5% level of significance, it was observed that base insignificantly ($0.05<p$) increased the skin melanin values while in case of formulation there was significant ($p\leq0.05$) decrease in skin melanin contents with respect to time. By applying paired sample *t*-test, it was observed that formulation presented significant ($p\leq0.05$) effects with respect to base. The decrease in skin melanin can be assigned to soy proteins; STI, a Kunitz-type trypsin inhibitor or soybean trypsin inhibitor, and BBI, the Bowman-Birk protease inhibitor. STI and BBI inhibit the protease-activated receptor 2 (PAR-2) activation and thus induce skin depigmentation by reducing the phagocytosis of melanosomes by keratinocytes, thus reducing melanin transfer (Paine et al., 2001) .Such agents may serve as an alternative, natural treatment for hyperpigmentation.

In case of cosmetic emulsion containing botanical extract of grape seeds the skin melanin contents of the volunteers was measured at specified intervals for 12 weeks. It showed markedly different effects of base and formulation on the skin melanin content. The base tends to increase the melanin level **(Table. 4.33)** whereas the formulation gradually decreased it and shown in table **(Table. 4.34 and Figure. 4.53)**. With the help of two-way ANOVA test defining a 5% level of significance, it was observed that base insignificantly ($0.05<p$) increased the skin melanin values while in case of formulation there was significant ($p\leq0.05$) decrease in skin melanin contents with respect to time. By applying paired sample *t*-test, it was observed that formulation presented significant ($p\leq0.05$) effects with respect to base.

The increase in melanin content by the base may be attributed to presence of paraffin oil. The preparations containing paraffin oil found to increase the melanin level in the skin when applied topically (Elleder and Borovanský, 2001). The reduction in the skin melanin content by the formulation may be credited to the Proanthocyanidins, which are oligomers and polymers of polyhydroxy flavan-3-ol units, such as catechin and epicatechin, are present in large amounts in the polyphenols of red wine and grape seeds. In grape seed extract (GSE), only the procyanidin type of proanthocyanidins has been detected. GSE is known as a powerful antioxidant that protects the body from premature aging, disease, and decay. Dietary supplements such as GSE enriched in OPCs (oligomeric procyanidins) have been suggested to have multiple health benefits, due to antioxidant and other beneficial activities of the plant extract (Zi et al., 2009). Grape seeds extract provides excellent protection against oxidative stress and free radical-mediated tissue injury. It has been reported that antioxidants or compounds with redox properties can inhibit or delay hyperpigmentation (Jiang et al., 2006).

Topical application of GSE enhances sun protection factor in human volunteers. It has been reported that proanthrocyanidines from GSE inhibited the activity of tyrosinases obtained from mushroom and from B16 mouse melanoma cells and decreased the melanin content of the cells. It is demonstrated that grape seeds extract has been effective in lightening the UV-induced pigmentation of guinea pig skin. This effect may be related to the inhibition of melanin synthesis by tyrosinase in melanocytes and the ROS-related proliferation of melanocytes (Yamakoshi et al., 2003).

In the case of cosmetic emulsion containing tamarind seeds extract, the base improves the skin melanin contents **(Table. 4.46)** but in case of test formulation there is regular decline in skin melanin values throughout the study duration **(Table. 4.47 and Figur. 4.62)**. With the help of two-way ANOVA test defining a 5% level of significance, it was observed that the base insignificantly ($0.05<p$) increased the skin melanin values while in case of formulation there was significant ($p \leq 0.05$) decrease in skin melanin contents with respect to time. By applying paired sample *t*-test, it was observed that formulation presented significant ($p \leq 0.05$) effects with respect to base. The increase in melanin content by the base product may be credited to presence of paraffin oil. The preparations containing paraffin oil found to increase the melanin level in the skin when applied topically (Elleder and Borovanský, 2001). The reduction in the skin melanin content by the formulation may be attributed to the presence of phenolic antioxidants, such as 2-hydroxy-30, 40-dihydroxyacetophenone, methyl 3, 4-dihydroxybenzoate, 3, 4-dihydroxyphenyl acetate and epicatechin. Extracts exhibit antioxidant potential by reducing lipid peroxidation in vitro. The content of Tamarind seeds comprised only procyanidins, represented (%) mainly by oligomeric procyanidin tetramer (30.2), procyanidin hexamer (23.8), procyanidin trimer (18.1), procyanidinpentamer (17.6)

with lower amounts of procyanidin B2 (5.5) and (-)-epicatechin (4.8) and possesses antioxidant activity(Thongmuang and Sudjaroen). It has been reported that antioxidant activity of these phenolic compounds is stronger than vitamin C and vitamin E .So they may reduce free radical and indirectly decrease melanin production. Proanthrocyanidines inhibited the activity of tyrosinases obtained from mushroom and from B16 mouse melanoma cells and decreased the melanin content of the cells. The further study needs to conduct to test the extracts with melanoma cell line (Parvez et al., 2007).

4.2.4.2. Skin erythema

For confirming the safety of cosmetics, the important point is that cosmetics must not cause any contact dermatitis when applied to the skin. The cause of contact dermatitis is not always due to cosmetic ingredients. Even if the safety of cosmetics is verified, it is known that environmental conditions such as temperature and humidity, misuse by the consumer, and the physical conditions may all cause contact dermatitis. Skin irritation is caused by the direct toxicity of chemicals on cells or blood vessels in the skin and is different from contact allergy which is caused by immune response (Nohynek et al., 2010).

Inflammation or skin irritation was continuously monitored every week for both the base and the formulation containing soybean seeds extract throughout the study period. Formulation purposive for external applications must not produce any redness or irritation and to evaluate it, patch test was carried out at the beginning of study which reflected that both base and formulation did not generate redness. In this research, it was observed that there was a uniform reduction in the values of skin erythema throughout the study duration after application of base samples. The formulation samples also displayed regular decline in erythema of skin from the start till the last week of the study. The percentage change was find out after 2nd, 4th, 6th, 8th, 10th and 12th week **(Table. 4.22-4.23 and Figure. 4.45)** ,which showed that was more reduction in erythema level expressed by the formulation. With the help of ANOVA test it was observed that base showed insignificant ($p \geq 0.05$) results on skin erythema with respect to time and temperature while formulation showed significant ($p \leq 0.05$) results on skin erythema with respect to time and temperature. By applying paired sample t-test, significant ($p \leq 0.05$) differences were noticed between the skin erythema of base and the formulation from the beginning of study duration. More declines in erythema level as compared to base may be due to the anti-inflammatory properties of soy bean extract.

Several studies dealing with the inhibition of inflammation by soy isoflavones are available in the literature. Several papers also suggested that genistein (a major soy isoflvone) could activate peroxisomal proliferator-activated receptor-γ (PPAR-γ) and in turn retard adhesion of monocyte to human vascular endothelial cells which may be associated with the A ring in the isoflavone structure The decline in erythema values represents that formulation tends to comfort and soothe the skin (Hämäläinen et al., 2007).

Skin irritation was continuously monitored every week for both the base and the formulation having grape seeds extract throughout the study period. It was found that there was irregular decline in the skin erythema contents after the application of base while formulation showed continuous decline throughout the study period **(Table. 4.35, 4.36 and Figure. 4.54)**. With the help of ANOVA test, it was evident that there were significant effects of base and formulation on skin erythema throughout the study period. With paired sample t test, it was found that the base and formulation produced insignificant results with respect to time regarding the skin erythema content after, 2nd, 4th, and 6th week while significant variations were shown after 6th week of the study. Different studies described that procyanidins (phenolic compounds from the flavonoids group) present in grape seeds extract have a broad range of biological activities. They function as powerful antioxidants and exert anti-inflammatory activities in vitro. Recent studies have shown potent anti-inflammatory properties of procyanidins on experimental inflammation in rats and mice. Its mechanisms of anti-inflammatory action remain poorly understood and are relevant to oxygen free radical scavenging, anti lipid peroxidation, inhibition of the formation of inflammatory cytokines, alterations in cell membranes receptors, intracellular signaling pathway proteins and modulation of gene expression (Li et al., 2001).

Skin irritation was also monitored every week for both the base and the formulation having tamarind seeds extract throughout the study period. It was found that there was irregular decline in the skin erythema contents after the application of base and formulation **(Table. 4.48, 4.49 and Figure. 4.63)**. With the help of ANOVA test, it was found that the base and formulation containing tamarind seeds extract produced insignificant result with respect to time. With the help of paired sample t test, no significance differences were observed between the erythema effects of base and the formulation.

4.2.4.3. Skin Moisture Contents

The moisture of the skin should be restored by preventing the loss of moisture in the stratum corneum layer. The increase in the moisture of stratum corneum supplies a smoother and more even look. Moisturizers protect the skin against external effects by forming a thin film layer on the skin. The moisturizing remedy involves renovating the skin barrier, restoring the water contents, ability to retain and redistribute water and sustaining the integrity and skin complexion (Baumann, 2008).

The effect of the base and formulation containing crude soybean seeds extract on skin hydration was examined. With the help of Corneometer MPA 5 (Courage and Khazaka GmbH, Germany), skin moisture contents were assessed before the application of creams (0 hour readings) and then at 2nd, 4th, 6th, 8th, 10th and 12th week of study duration. For eleven volunteers, the percentage changes in readings were determined. In this work base enhances the moisture contents of skin to some degree but there was consistent increase in skin moisture contents when formulation was applied throughout the study duration **(Table. 4.24-4.25 and Figure. 4.46)**. By applying two-way ANOVA insignificant effects ($p>0.05$) were observed by the base while the formulation presented significant ($p\leq0.05$) effects on skin moisture contents regarding with time. Throughout the study duration significant differences in moisture values were evaluated after application of formulation by applying paired sample t-test. Significant differences in moisture values were evaluated after application of formulation throughout study duration.

The enhancement of skin moisture contents after the application of formulation may because of soy bean extract which contain isoflavonoids (quercetin, genistein, daidzein). Soybean iso flavonones enhances the skin moisture contents due to swelling of corneocytes on skin surface. Consequently, it may be used in moisturizing cosmetic formulations and also as a complement in the treatment of dry skin (Lazarus and Baumann, 2001). In case of increase in hydration level of the stratum corneum by base, the mineral oil (Paraffin oil) may be attributed as it is described in the literature that vehicles like mineral oils (Paraffin oil) exhibit occlusive softening effect on the human skin by protecting water loss from stratum corneum (Sato et al., 2002).

In case of emulsions containing grape and tamarind seeds extracts, a regular increase in the moisture content after application of formulation but the effect of the base was not regular

(Table. 4.37-4.38, 4.50-4.51 and Figure. 4.55, 4.64). The ANOVA test showed that effect of formulation was significant ($p \leq 0.05$) whereas the base has insignificant ($p > 0.05$) effects on the skin moisture content with respect to time. Moreover, the paired sample t test proved that there had been significant differences in moisture values at all the specified intervals. Both grape and tamarind seeds extract contain high concentration of proanthrocyanidines(Ling and Bai-ping, 2008, Siddhuraju, 2007). Proanthrocyanidines (PAs) were found to increase collagen synthesis and accelerate the conversion of soluble collagen to insoluble collagen during development and decrease the rate of enzymatic degradation of collagen matrices (Han et al., 2003). It is reported that as the collagen intensity is increased, the hydration level also elevated (Sharma et al., 2008).

4.2.4.4. Skin Elasticity

The elasticity of the skin is mainly due to the collagen fibers which is a main constituent of the dermis of the skin. It constitutes about 70% of total dry weight of the skin. Vitamin C stimulates the collagen production in the skin fibroblasts and its deficiency will result in harmful effects on the skin. The collagen is vulnerable to the UV radiations of the sunlight which causes structural changes in them resulting in photo aging. Photo aging is due to the oxidative effects of these radiations on the collagen of the skin(Yilmaz and Borchert, 2006).

The effect of the base and formulation containing crude soybean seeds extract on the skin elasticity of the volunteers was measured with the help of skin Elastometer®EM 25(Courage and Khazaka GmbH, Germany) at specified intervals for a period of 12 weeks. The irregular pattern in the values of skin elasticity was observed by the base but there was regular increase in skin elasticity after the application of formulation throughout the study duration **(Table. 4.26, 4.27 and Figure. 4.47)**. By the use of ANOVA two way analysis it was observed that formulation had significant ($p<0.05$) while the base had insignificant ($p \geq 0.05$) effects on skin elasticity. By applying paired sample *t*-test it was obvious that significant differences in skin elasticity values were noticed after application of the formulation throughout the study duration.

The improvement in skin elasticity after application of formulation is due to the presence phytoestrogens (Tempfer et al., 2007). Soybean extracts was found to investigate the synthesis of collagen and elastin, and to boost the correct assembly of new elastin fibers, providing a complete protection and restoration to the dermal extracellular matrix (Draelos, 2005). Soybeans extract also protected elastic fibers produced by cultured fibroblasts from degradation by exogenously-added elastases (Waqas et al., 2014).

The effects of the cosmetic emulsions containing botanical extracts of grape and tamarind seeds on the skin elasticity of the volunteers was measured at specified intervals for 12 weeks. The results showed an increase in the skin elasticity with formulation **(Table. 4.39-4.40, 4.54-4.55 and Figure. 4.56, 4.66)** The ANOVA test proved that the variations in values of skin elasticity by formulation was significant whereas with base it was insignificant ($p\leq 0.05$) with respect to time. With paired sample *t* test, it was evident that formulation presented significant ($p\leq0.05$) effects with respect to base.

The improvement in skin elasticity by grape seeds extract can be attributed to the phenolic compounds and flavanoids present in grape seeds extract which include resveratrol,catechins ,epicatechin, epicatechin-3-O-gallate, procyanidins dimers (B1-B5), procyanidin C1, and procyanidin B5-3′-gallate kaempferol-3-O-glucosides, quercetin-3-Oglucosides, quercetin , myricetin (Shi et al., 2003, Alarcón de la Lastra and Villegas, 2005).

While tamarind seeds extract contains phenolic antioxidants such as 2-hydroxy-30, 40-dihydroxyacetophenone, methyl 3,4-dihydroxybenzoate, 3,4-dihydroxyphenyl acetate and epicatechin, procyanidins, represented mainly by oligomeric procyanidin tetramer , procyanidin hexamer , procyanidin trimer , procyanidinpentamer with lower amounts of procyanidin B2 and epicatechin (Siddhuraju, 2007).

Proanthocyanidins (PAs) improves the skin elasticity by increasing the collagen synthesis and decreasing the rate of enzymatic degradation of collagen matrices(Yokozawa et al., 2011). PAs can also inhibit the catabolism of soluble collagen in animal studies. It stimulates normal skin fibroblast proliferation, and increase the synthesis of extracellular matrix, including collagen and fibronectin. PAs are natural products with polyphenolic structures that have the potential to give rise to stable hydrogen bonded structures and generate collagen matrices (Han et al., 2003).

4.2.4.5. Skin Sebum Contents

Sebaceous glands are multilobular holocrine glands generally associated with hair follicles. The basal sebocytes sit on a basal membrane at the outer limits of the lobes, and as cells move from the basal layer toward the lumen of the gland they synthesize lipids, which accumulate as intracellular lipid droplets. As they synthesize lipid, the cells become larger, and the nucleus and other internal organelles are degraded. Ultimately, the entire mass of the cell is converted into a viscous liquid phase lipid mixture. In most pilosebaceous units, sebum passes from the sebaceous gland into the hair follicle via the short sebaceous duct and outward onto the skin surface through the follicle. Generally, the hair follicle is large compared with the associated sebaceous gland; however, large sebaceous glands are associated with vellous hairs. These units are called sebaceous follicles and predominate on the forehead and cheeks (Zouboulis, 2004). Human sebum from isolated sebaceous glands consists mainly of squalene, wax esters, and triglycerides with small proportions of cholesterol and cholesterol esters. There is a clear positive correlation between the occurrence and severity of acne and the sebum secretion rate. It has been suggested that the development of acne may result from essential fatty acid deficiency localized to the follicular epithelium (Simpson and Cunliffe, 2004). In experimental systemic essential fatty acid deficiency, the skin becomes scaly and more permeable. If sebaceous fatty acids were to penetrate into the follicular epithelial cells and compete with linoleic acid from the circulation for incorporation into lipids, a localized essential fatty acid deficiency could be produced. The resulting scaling could lead to comedo formation, and the defective barrier function would facilitate exchange of materials between the follicle and surrounding tissue. This would include an influx of water and nutrients into the follicle to support bacterial growth as well as the efflux of inflammatory mediators (Yosipovitch et al., 2007).

The effect of the base and formulation containing soybean seeds extract on skin sebum contents was determined with the help of a photometric device Sebumeter MPA 5(Courage and Khazaka GmbH, Germany).Sebum was collected with special opaque plastic tape (64 mm^2) by pressing on to the skin surface for 30 seconds with a slight pressure. As sebum is secreted from the orifice of a follicle, it is adsorbed into the pores in the polymer, and this turns the appearance of the tape from opaque to transparent. The light transmission represents the sebum content on the surface of the measuring area. A microprocessor calculates the result, which is shown on the display in values from 0–350 (O'goshi, 2006). In this study the increased sebum contents was observed by the base throughout the study period of 12 weeks. The increase in sebum contents by base may be due to the oily nature of emulsion, as mineral oil (Paraffin oil) was used, which has the property to increase the sebum content of the skin (Abramovits and Gonzalez-Serva, 2000).

In case of the formulation containing soybean seeds extract , a regular decline was observed in the sebum contents throughout the study period of 12 weeks **(Table. 4.28, 4.29 and Figure. 4.48)**.By applying ANOVA test, it was found that base increased sebum insignificantly ($p > 0.05$) while the formulation had a significant ($p \leq 0.05$) decrease with respect to time. Paired sample t-test showed that formulation produced significant effects in comparison with base.

Formulation containing grape seeds extract was also evaluated for skin sebum level. Sebum contents were measured in each individual at different time intervals for the period of 12 weeks. It was found that the base showed a variable increasing tendency on skin sebum content**,** while the formulation showed a decrease in sebum contents throughout the study period **(Table. 4.41, 4.42 and Figure. 4.57)**. With the help of ANOVA test it was found that the base produced insignificant while formulation produced significant effects on skin sebum contents with respect to time**.** Significant differences were observed between the sebum effects of base and that of formulation when paired sample *t*-test was applied. Different types of phenolic compounds *i.e.* genistein,catechins; epicatechin, epicatechin-3-O-gallate, procyanidins dimers (B1-B5), procyanidin C1, and procyanidin B5-3′-gallate are present in soy bean and grape seeds respectively (Malenčić et al., 2008, Nawaz et al., 2006).

Peroxisome proliferator activated receptors (PPAR) are known nuclear hormone receptors having the three isoforms of α, β/δ and γ. It is demonstrated that such activators enhance formation of an epithelial barrier *in vitro*. Additionally, it is reported that PPAR-γ affects maturation of sebocytes in the sebaceous gland. It was observed in a study that cosmetic composition containing polyphenols such as catechins, epicatechin and epicatechin-3-O-gallate,genistein suppress the sebum generation by reducing the activity of (PPAR - γ) that affects the maturation of sebocytes in sebaceous gland (Ali et al., 2012b).

The effects of the base and formulation containing tamarind seeds extract on sebum contents of volunteer skin were also evaluated for a period of 12 weeks. Skin sebum content was determined at intervals of 2nd, 4th, 6th, 8th, 10th and 12th week and percentage of changes are represented in **(Table. 4.52, 4.53 and Figure. 4.65)**. In this study, the base increased the skin sebum content which can be due to oily nature of base having paraffin oil in it, but in case of formulation there was decline observed in skin sebum content throughout the study period. By applying ANOVA test, it was found that base increased sebum insignificantly ($p > 0.05$) while the formulation had a significant ($p \leq 0.05$) decrease with respect to time. Paired sample t-test showed that formulation produced significant effects in comparison with base.

The decline in sebum contents shown by the formulation containing tamarind seeds extract can be attributed due to the presence of unsaturated fatty acids present in tamarind seeds which include linoleic acid and oleic acid (De Caluwé et al., 2010). Topical application of linoleic acid has shown to inhibit sebum production due to selective inhibition of 5α-reductase, an enzyme found in sebaceous glands responsible for sebum production. There are two isozymes, type1 and type 2 for 5α-reductase. The linoleic acid suppresses both types (Akhtar et al., 2010a).

4.2.4.6. Surface Evaluation of Living Skin (SELS)

The values of different SELS parameters SE*r*, SE*sc*, SE*sm* and SE*w* was measured by Visioscan® VC 98/ software SELS 2000 (Courage and Khazaka GmbH, Germany) before application of emulsions (0 hour readings) and at 1st, 2nd and 3rd month of study period. *SEr* is the roughness parameter which depicts the asperity of the skin and calculates the gray levels above the threshold in comparison with the whole image. It calculates the proportion of dark pixels. *SEsm* is the index of smoothness and is calculated from mean width and depth of wrinkles. *SEsc* is the index representing scaliness of skin which shows the level of dryness of the stratum corneum i.e., state of dehydration of the skin. It is the number of pixels where the gray level is higher than the threshold of *SEsc*. *SEw* identifies aging including wrinkles and is calculated from the proportion of horizontal and vertical wrinkles (Khan et al., 2012).

In this study all four parameters of SELS are improved by the application of cosmetic emulsions containing the botanical extracts of soybean, grape and tamarind seeds respectively. The cosmetic emulsions showed decrease in mean values of skin roughness (SEr) which indicated that the formulations possess anti-aging properties. A decline in the values of the skin wrinkles (*SEw)* was observed for cosmetic emulsions containing soybean, tamarind and grape seeds extract**(Table. 4.30, 4.43, 4.56)**. The perfection in SELS parameters can be attributed to the polyphenolic flavonoids present in these plants. This is also obvious from the figures(**Figure. 4.49-4.52, 4.58-4.61 and 4.67-4.70)** where the visible differences can be seen between the peaks of the 3D image. The formulations showed decrease in mean values of skin smoothness in contrast to skin roughness which indicates that the formulations possess anti-aging properties. Lower values of skin scaliness showed that the skin got hydrated with the passage of time during treatment. It was also supported by the values obtained by Corniometer® MPA 5 for skin hydration. The smaller *SEsc* value corresponds to higher skin moisture as treatment with moisturizing or anti-aging formulations let the values for *SEsc* go down. Over exposure to the sun light results in many of the skin changes associated with aging characterized by fine wrinkling and skin laxity. Collagen is responsible for imparting the skin pliability and elasticity. Decreased levels of collagen occur in both chronologic and photoaging. As collagen fibers serve as the primary structural support of the skin, it follows logically that a reduction in skin collagen levels would be associated with the formation of skin wrinkles.

The improvement in skin surface parameters by cosmetic emulsion containing soybean seeds extract is due to the existence of rich source of antioxidant flavonoids, known as genistein and daidzein in soybeans. These substances have been classified as phytoestrogens, since they are plant derivatives with chemical structure similar to human estrogen. Topical estrogens have been shown to function as antiaging cosmeceuticals by increasing skin thickness, promoting the collagen synthesis and prevention of skin from oxidative damage and photoaging (Draelos, 2005).

The improvement in skin surface parameters by cosmetic emulsions containing grape and tamarind seeds extract can be attributed to the phenolic compounds and flavanoids which include catechins ,epicatechin, epicatechin-3-O-gallate, procyanidins dimers (B1-B5), procyanidin C1, and procyanidin B5-3′-gallate kaempferol-3-O-glucosides, quercetin-3-Oglucosides, quercetin , myricetin. There is much supporting evidence present that phenolic compounds contribute towards antiaging mechanism by providing an antioxidative benefit to skin. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and oxygen quenchers. They represent a class of compounds comprising of potential candidates for prevention of the adverse effects of UV radiation on the skin. Proanthrocyanidines present in grape and tamarind seeds extract have been shown to exert a much stronger oxygen free radical scavenging effects than vitamin C and E and to prevent UVB- and UVC-induced lipid peroxidation (Shi et al., 2003, El Gharras, 2009).

CHAPTER 5

CONCULSION AND FUTURE PERSPECTIVES

5. Overall conclusions and Future perspectives

5.1. Overall conclusions

It can be summarized from this research work that;

- Cosmetic emulsions containing botanical extracts used in this study i.e., soy beans (*Glycin max*), Grape seeds (*Vitis vinifera*) and Tamarind seeds (*Tamarindus indica*) show potential skin whitening and anti-aging properties when applied topically and stable topical cosmetic emulsions containing theses botanical extracts can be formulated.
- The active formulation containing botanical extracts were evaluated for possible antiaging effects as these displayed good antioxidant activities when DPPH assay was performed.
- The active formulations (SF, GF and TF) caused increase in skin moisture, skin elasticity and decrease in skin sebum contents when measured quantitatively by Corneometer® MPA 5, Sebumeter® MPA 5 and Skin Elastometer®EM 25 proving that selected botanical extracts have positive effects when applied topically to human skin especially in dry skin, acne and photoaging treatment.
- Non-invasive *in-vivo* evaluation of the prepared cosmetic emulsions produced some superior effects on surface evaluation of the living skin (SELS) parameters i.e., *SEr* (skin roughness), *SEsm* (skin smoothness), *SEsc* (skin scaliness) and *SEw* (skin wrinkles).These parameters were observed to have declining values along the course of study during a three months period depicting that the formulations possess anti-wrinkle effects.
- Corresponding bases (SB, GB, TF) used as control were observed to have varying but insignificant effects on various skin physiological parameters.

- The further studies such as irritability to skin, skin rejuvenation efficiency, and acceptance from the volunteers/consumers have evaluated the value of our prepared cosmetic emulsions. None of the emulsions was found irritant to the skin.

- These findings provide scientific evidence that noninvasive biophysical techniques are a valuable tool for the evaluation of cosmeceutical effects of topical emulsions containing various botanical extracts.

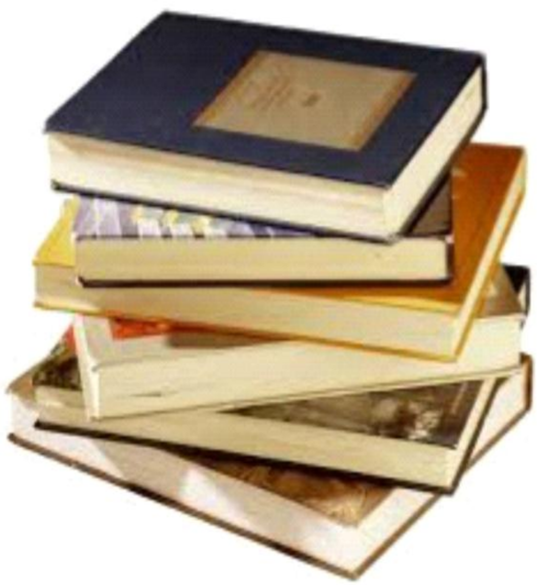
5.2. FUTURE PERSPECTIVES

Despite the fact that adequate data has been displayed in this research work regarding the formulation development of cosmetic emulsions loaded with various botanical extracts with good shelf-storage stability, still some other studies could be presented like:

- ❖ Formulation development of cosmetic emulsions with isolated bioactive components of the botanical extracts. However for cosmetic industry, isolation and purification of the active ingredient from the crude extract are sometimes not desirable because such isolation and purification may lead to loss in the biological activity.
- ❖ The cosmetic emulsions evaluated in this study carried no preservatives. Long term stability of the emulsions can be assured by adding preservatives and stability testing for increased duration.
- ❖ Other types of dosage forms like micro-emulsion, multiple emulsion, gel, ointment and lotion for topical skin formulations can be formulated, while incorporating these botanical extracts.
- ❖ *In-vitro* skin permeability studies of the cosmetic emulsions may also be performed.

CHAPTER 6

REFERANCES



6. Referances

ABRAMOVITS, W. & GONZALEZ-SERVA, A. 2000. Sebum, cosmetics, and skin care. *Dermatologic clinics*, 18**,** 617-620.

AFAQ, F., ADHAMI, V. M., AHMAD, N. & MUKHTAR, H. 2002. Botanical antioxidants for chemoprevention of photocarcinogenesis. *Frontiers in bioscience: a journal and virtual library,* 7**,** d784-92.

AFAQ, F. & MUKHTAR, H. 2006. Botanical antioxidants in the prevention of photocarcinogenesis and photoaging. *Experimental dermatology,* 15**,** 678-684.

AFRIAT, I. & CHANVIN, F. 2002. Emulsion containing ascorbic acid and its uses in the cosmetics and dermatological fields. Google Patents.

AGARWAL, S. P. 2007. Identification tests for emulsions. In Physical Pharmacy 2nd edn ed.: CBS P ublishers.

AKAZAKI, S., NAKAGAWA, H., KAZAMA, H., OSANAI, O., KAWAI, M., TAKEMA, Y. & IMOKAWA, G. 2002. Age-related changes in skin wrinkles assessed by a novel three-dimensional morphometric analysis. *British Journal of Dermatology,* 147**,** 689-695.

AKHTAR, N., KHAN, B., KHAN, M., MAHMOOD, T., KHAN, H., IQBAL, M. & BASHIR, S. 2011. Formulation development and moiturising effects of a topical cream of Aloe vera extract. *World Academy of Science, Engineering and Technology,* 51**,** 172-180.

AKHTAR, N., KHAN, B. A., MAHMOOD, T., PARVEEN, R., QAYUM, M. & ANWAR, M. 2010a. Formulation and evaluation of antisebum secretion effects of sea buckthorn w/o emulsion. *Journal of Pharmacy And Bioallied Sciences,* 2**,** 13.

AKHTAR, N., KHAN, H. M. S., GULFISHAN, RASOOL, F., AHMAD, M. & SAEED, T. 2010b. Formulation and in vitro evaluation of a cosmetic emulsion containing apple juice extract. *ASIAN JOURNAL OF CHEMISTRY,* 22**,** 7235-7242.

AKHTAR, N., WAQAS, M., AHMED, M., ALI, A., SAEED, T., MURTAZA, G., RASOOL, A., AAMIR, M., KHAN, S. & BHATTI, N. 2010c. Effect of cream formulation of fenugreek seed extract on some mechanical parameters of human skin. *Tropical Journal of Pharmaceutical Research,* 9.

ALARCÓN DE LA LASTRA, C. & VILLEGAS, I. 2005. Resveratrol as an anti-inflammatory and anti-aging agent: Mechanisms and clinical implications. *Molecular nutrition & food research,* 49**,** 405-430.

ALI, A., AKHTAR, N. & KHAN, H. M. S. 2012a. Assessment of Physical Stability and Antioxidant Activity of Polysiloxane Polyalkyl Polyether Copolymer-Based Creams. *Journal of Chemistry,* 2013.

ALI, A., AKHTAR, N. & SHOAIB KHAN, H. M. 2012b. Cream Containing Acacia Bark Extract Significantly Reduces Skin Sebum Content in Healthy Volunteers. *Journal of Scientific and Industrial Research,* 71**,** 678.

ANTIGNAC, E., NOHYNEK, G. J., RE, T., CLOUZEAU, J. & TOUTAIN, H. 2011. Safety of botanical ingredients in personal care products/cosmetics. *Food and Chemical Toxicology,* 49**,** 324-341.

ASHOK, B. T. & ALI, R. 1999. The aging paradox: free radical theory of aging. *Experimental gerontology,* 34**,** 293-303.

ASHOK KG, B. S. 2008. *Introduction to Pharmaceutics-II.* , CBS Delhi.

B, P. 2005. *Remington the Science and Practice of Pharmacy*, Lippincott Williams and Wilkins, Philadelphia.

BAGCHI, D., GARG, A., KROHN, R., BAGCHI, M., BAGCHI, D., BALMOORI, J. & STOHS, S. 1998. Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. *General Pharmacology: The Vascular System,* 30**,** 771-776.

BAQUIÉ, M. & KASRAEE, B. 2014. Discrimination between cutaneous pigmentation and erythema: Comparison of the skin colorimeters Dermacatch and Mexameter. *Skin Research and Technology,* 20**,** 218-227.

BARBARA Y, J. W. 2000. *Wheater Functional Histology*, Harcourt publishers limited, London.

BAREL, A. O. & CLARYS, P. 1997. In vitro calibration of the capacitance method (Corneometer CM 825) and conductance method (Skicon-200) for the evaluation of the hydration state of the skin. *Skin Research and Technology,* 3**,** 107-113.

BAUMANN, L. 2004. Cosmeceutical Critique. Grapeseed. *Skin & Allergy News,* 26.

BAUMANN, L. 2008. Cosmetics and skin care in dermatology. *Wolff K, et al. Fitzpatrick's Dermatology in General Medicine. 7th ed. New York, NY: The McGraw-Hill Companies*.

BAUMANN, L. S. 2001. Cosmeceutical critique: soy and its isoflavones. *Skin & Allergy News,* 32**,** 17.

BHARGAVA, H. 1987. The present status of formulation of cosmetic emulsions. *Drug Development and Industrial Pharmacy,* 13**,** 2363-2387.

BINKS, B. & LUMSDON, S. 2000. Catastrophic phase inversion of water-in-oil emulsions stabilized by hydrophobic silica. *Langmuir,* 16**,** 2539-2547.

BINKS, B. P., CLINT, J. H. & WHITBY, C. P. 2005. Rheological behavior of water-in-oil emulsions stabilized by hydrophobic bentonite particles. *Langmuir,* 21**,** 5307-5316.

BIRBECK, M. S., BREATHNACH, A. S. & EVERALL, J. D. 1961. An electron microscope study of basal melanocytes and high-level clear cells (Langerhans cells) in vitiligo1. *Journal of Investigative Dermatology,* 37**,** 51-64.

BLECKMANN, A., KRÖPKE, R. & SCHNEIDER, G. 2006. Preparations of the W/O emulsion type with an increased water content, and comprising cationic polymers. Google Patents.

BOISSIEUX, L., KISS, G., THALMANN, N. M. & KALRA, P. 2000. *Simulation of skin aging and wrinkles with cosmetics insight*, Springer.

BOODE, K. & WALSTRA, P. 1993. Partial coalescence in oil-in-water emulsions 1. Nature of the aggregation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 81**,** 121-137.

BOUCHEMAL, K., BRIANÇON, S., PERRIER, E. & FESSI, H. 2004. Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimisation. *International Journal of Pharmaceutics,* 280**,** 241-251.

BOUHAMIDI, R., PRÉVOST, V. & NOUVELOT, A. 1998. High protection by grape seed proanthocyanidins (GSPC) of polyunsaturated fatty acids against UV-C induced peroxidation. *Comptes Rendus de l'Académie des Sciences-Series III-Sciences de la Vie,* 321**,** 31-38.

BOYD, J. V., PARKINSON, C. & SHERMAN, P. 1972. Factors affecting emulsion stability, and the HLB concept. *Journal of Colloid and Interface Science,* 41**,** 359-370.

BRIGANTI, S., CAMERA, E. & PICARDO, M. 2003. Chemical and instrumental approaches to treat hyperpigmentation. *Pigment Cell Research,* 16**,** 101-110.

CADENAS, E. & DAVIES, K. J. 2000. Mitochondrial free radical generation, oxidative stress, and aging. *Free Radical Biology and Medicine,* 29**,** 222-230.

CALABRESE, V. & MAINES, M. D. 2006. Antiaging medicine: antioxidants and aging. *Antioxidants & redox signaling,* 8**,** 362-364.

CARTER, S. J. 2007. *Instability of emulsions. In Dispensing for Pharmaceutical Students* ,

Delhi, CBS Publishers and Distributers.

CHAKRABORTY, A. K., FUNASAKA, Y., SLOMINSKI, A., ERMAK, G., HWANG, J., PAWELEK, J. M. & ICHIHASHI, M. 1996. Production and release of proopiomelanocortin (POMC) derived peptides by human melanocytes and keratinocytes in culture: regulation by ultraviolet B. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research,* 1313**,** 130-138.

CHANG, S. S. & LINDMARK, L. 1986. Oil-in-water emulsion for parenteral administration. Google Patents.

CHATURVEDI, S., SHARMA, P., GARG, V. K. & BANSAL, M. 2011. Role of Nutraceuticals in health promotion. *health,* 4**,** 5.

CHOI, M. H., JEONG, S., NAM, S. I., SHIM, S. E. & CHANG, Y. H. 2009. Rheology of decamethylceclopentasiloxane (cyclomethicone) W/O emulsion system. *Macromolecular Research,* 17**,** 943-949.

CHU, L. Y., UTADA, A. S., SHAH, R. K., KIM, J. W. & WEITZ, D. A. 2007. Controllable monodisperse multiple emulsions. *Angewandte Chemie International Edition,* 46**,** 8970-8974.

CLARK, C. 2004. How to choose a suitable emollient. *Pharmaceutical journal,* 273**,** 351-353.

CLARYS, P., ALEWAETERS, K., LAMBRECHT, R. & BAREL, A. 2000. Skin color measurements: comparison between three instruments: the Chromameter®, the DermaSpectrometer® and the Mexameter®. *Skin research and Technology,* 6**,** 230-238.

COURAGE, W. & KHAZAKA, G. 1988. Instruction Manual for Sebumeter SM 410. *Courage and Khazaka: Cologne, Federal Republic of Germany*.

CRONIN, H. & DRAELOS, Z. D. 2010. Original Contribution: Top 10 botanical ingredients in 2010 anti-aging creams. *Journal of cosmetic dermatology,* 9**,** 218-225.

DE CALUWÉ, E., HALAMOVÁ, K. & VAN DAMME, P. 2010. Tamarindus indica L.: a review of traditional uses, phytochemistry and pharmacology. *Afrika focus,* 23**,** 53-83.

DITRE, C., WU, J., BAUMANN, L. S. & RIGEL, D. 2008. Innovations in natural antioxidants and their role in dermatology. *Cutis,* 82**,** 2-16.

DO PRADO, D. Z., STAPENHORST, A., SIEGA, C. & DE SOUZA, J. S. 2013. Skin Evaluation Systems. *Update in Cosmetic Dermatology.* Springer.

DRAELOS, Z. D. 2005. Topical and oral estrogens revisited for antiaging purposes. *Fertility and sterility,* 84**,** 291-292.

ECCLESTON, G. M. 1997. Formulating cosmetic emulsions. *Cosmetics and toiletries,* 112**,** 65-72.

EDWARDS, R. D. & LIOY, P. J. 2001. Influence of sebum and stratum corneum hydration on pesticide/herbicide collection efficiencies of the human hand. *Applied occupational and environmental hygiene,* 16**,** 791-797.

EL-ASHMAWY, I. M., SALEH, A. & SALAMA, O. M. 2007. Effects of marjoram volatile oil and grape seed extract on ethanol toxicity in male rats. *Basic & clinical pharmacology & toxicology,* 101**,** 320-327.

EL GHARRAS, H. 2009. Polyphenols: food sources, properties and applications–a review. *International journal of food science & technology,* 44**,** 2512-2518.

ELLEDER, M. & BOROVANSKÝ, J. 2001. Autofluorescence of melanins induced by ultraviolet radiation and near ultraviolet light. A histochemical and biochemical study. *The Histochemical Journal,* 33**,** 273-281.

ELMALI, N., ESENKAYA, İ., KARADAĞ, N. & TAŞ, F. 2007. Effects of resveratrol on skeletal muscle in ischemia-reperfusion injury. *Ulusal travma ve acil cerrahi dergisi= Turkish journal of trauma & emergency surgery: TJTES,* 13**,** 274-280.

FANG, Y.-Z., YANG, S. & WU, G. 2002. Free radicals, antioxidants, and nutrition. *Nutrition,* 18**,** 872-879.

FISHER, G. J. 2005. The pathophysiology of photoaging of the skin. *Cutis,* 75**,** 5-8; discussion 8-9.

FISHER, G. J., KANG, S., VARANI, J., BATA-CSORGO, Z., WAN, Y., DATTA, S. & VOORHEES, J. J. 2002. Mechanisms of photoaging and chronological skin aging. *Archives of dermatology,* 138**,** 1462-1470.

FLORENCE, A. & WHITEHILL, D. 1982. The formulation and stability of multiple emulsions. *International Journal of Pharmaceutics,* 11**,** 277-308.

FLOURY, J., DESRUMAUX, A. & LARDIERES, J. 2000. Effect of high-pressure homogenization on droplet size distributions and rheological properties of model oil-in-water emulsions. *Innovative Food Science & Emerging Technologies,* 1**,** 127-134.

FLUHR, J. W., GLOOR, M., LAZZERINI, S., KLEESZ, P., GRIESHABER, R. & BERARDESCA, E. 1999. Comparative study of five instruments measuring stratum corneum hydration (Corneometer CM 820 and CM 825, Skicon 200, Nova DPM 9003, DermaLab). Part I. In vitro. *Skin Research and Technology,* 5**,** 161-170.

FOMUSO, L. B., CORREDIG, M. & AKOH, C. C. 2002. Effect of emulsifier on oxidation properties of fish oil-based structured lipid emulsions. *Journal of agricultural and food chemistry,* 50**,** 2957-2961.

FRANCKUM, J. P., RAMSAY, D. P., DAS, N. & DAS, S. 2003. Pluronic Lecithin Organogel for Local Delivery of Anti-Inflammatory Drugs. *International journal of pharmaceutical compounding,* 8**,** 101-105.

FU, C., LOO, A. E. K., CHIA, F. P. P. & HUANG, D. 2007. Oligomeric proanthocyanidins from mangosteen pericarps. *J. of Agricultural and Food Chemistry,* 55**,** 7689-7694.

GHELARDI, E., TAVANTI, A., DAVINI, P., CELANDRONI, F., SALVETTI, S., PARISIO, E., BOLDRINI, E., SENESI, S. & CAMPA, M. 2004. A mucoadhesive polymer extracted from tamarind seed improves the intraocular penetration and efficacy of rufloxacin in topical treatment of experimental bacterial keratitis. *Antimicrobial agents and chemotherapy,* 48**,** 3396-3401.

GOTO, M., SUMURA, H., ABE, K. & NAKASHIO, F. 1995. Novel preparation method for surfactant-coated enzymes using W/O emulsion. *Biotechnology techniques,* 9**,** 101-104.

GRABE, N. & NEUBER, K. 2005. A multicellular systems biology model predicts epidermal morphology, kinetics and Ca2+ flow. *Bioinformatics,* 21**,** 3541-3547.

GRAF, J. 2010. Antioxidants and skin care: The essentials. *Plastic and reconstructive surgery,* 125**,** 378-383.

GRUENWALD, J., BRENDLER, T., JAENICKE, C., FLEMING, T., DEUTSCH, M. & HAMID, M. 1998. PDR for Herbal Medicines. Montvale, NJ: Medical Economics Company. Inc.

HÄMÄLÄINEN, M., NIEMINEN, R., VUORELA, P., HEINONEN, M. & MOILANEN, E. 2007. Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-κ B activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-κ B activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. *Mediators of inflammation,* 2007.

HAN, B., JAUREQUI, J., TANG, B. W. & NIMNI, M. E. 2003. Proanthocyanidin: a natural crosslinking reagent for stabilizing collagen matrices. *Journal of Biomedical Materials Research Part A,* 65**,** 118-124.

HARMAN, D. 2006. Free radical theory of aging: an update. *Annals of the New York Academy of Sciences,* 1067**,** 10-21.

HEMMI, H., YOSHINO, M., YAMAZAKI, H., NAITO, M., IYODA, T., OMATSU, Y., SHIMOYAMA, S., LETTERIO, J. J., NAKABAYASHI, T. & TAGAYA, H. 2001. Skin antigens in the steady state are trafficked to regional lymph nodes by transforming growth factor-β1-dependent cells. *International immunology,* 13**,** 695-704.

HENDRIKS, F., BROKKEN, D., OOMENS, C., BADER, D. & BAAIJENS, F. 2006. The relative contributions of different skin layers to the mechanical behavior of human skin in vivo using suction experiments. *Medical engineering & physics,* 28**,** 259-266.

HERBERT A. L., M. M. R. A. G. S. B. 1989. *Pharmaceutical Emulsions, Pharmaceutical Dosage Forms: Disperse Systems,* Marcel Dekker, New York and Basel.

HERBERT A. L., M. M. R. A. G. S. B. 2005. *Pharmaceutical Emulsions, Pharmaceutical Dosage Forms: Disperse Systems,* Marcel Dekker, New York and Basel.

HERBERT, W. 1965. Multiple emulsions: a new form of mineral-oil antigen adjuvant. *The Lancet,* 286**,** 771.

HO, H. O., HSIAO, C. C. & SHEU, M. T. 1996. Preparation of microemulsions using polyglycerol fatty acid esters as surfactant for the delivery of protein drugs. *Journal of pharmaceutical sciences,* 85**,** 138-143.

HOLICK, M. F., CHEN, T. C., LU, Z. & SAUTER, E. 2007. Vitamin D and Skin Physiology: AD-Lightful Story. *Journal of Bone and Mineral Research,* 22**,** V28-V33.

HUANG, L.-H., SHIAO, N.-H., HSUUW, Y.-D. & CHAN, W.-H. 2007. Protective effects of resveratrol on ethanol-induced apoptosis in embryonic stem cells and disruption of embryonic development in mouse blastocysts. *Toxicology,* 242**,** 109-122.

İBANOĞLU, E. 2002. Rheological behaviour of whey protein stabilized emulsions in the presence of gum arabic. *Journal of Food Engineering,* 52**,** 273-277.

J, N. G. 2006. *Skin, A Natural History*, University of California Press, USA.

JANSEN, B. J. & SCHALKWIJK, J. 2003. Transcriptomics and proteomics of human skin. *Briefings in functional genomics & proteomics,* 1**,** 326-341.

JELLIN, J. M., GREGORY, P. & BATZ, F. 2000. *Natural medicines comprehensive database*, Stockton, CA: Therapeutic Research Faculty 1521p. ISBN.

JIANG, C., CHANG, M., WEN, C., LIN, Y., HSU, F. & LEE, M. 2006. Natural products of cosmetics: analysis of extracts of plants endemic to Taiwan for the presence of tyrosinase-inhibitory, melanin-reducing, and free radical scavenging activities. *Journal of Food and Drug Analysis,* 14**,** 346.

JIANG, L. C., BASRI, M., OMAR, D., RAHMAN, M. B. A., SALLEH, A. B. & RAHMAN, R. N. Z. R. A. 2011. Physicochemical Characterization of Nonionic Surfactants in oil-in-water (O/W) Nano-emulsions for New Pesticide Formulations. *International Journal of Applied,* 1.

JINDAL, V., DHINGRA, D., SHARMA, S., PARLE, M. & HARNA, R. K. 2011. Hypolipidemic and weight reducing activity of the ethanolic extract of Tamarindus indica fruit pulp in cafeteria diet-and sulpiride-induced obese rats. *Journal of pharmacology & pharmacotherapeutics,* 2**,** 80.

JOHN, E. H. 1976. *Dispensing Of Medication*, Mack Publishing Company Pennsylvania.

K, M. D. 2006. *Using organotypic raft cultures to understand the biological basis of Raman spectra from skin.* Thesis Submitted to the Faculty of the Graduate School of Vanderbilt University.

K SURIA P, D. M. G. 2008. *Pharmacy Practice and Pathophysiology* Birala, Delhi.

KABALNOV, A. & WENNERSTRÖM, H. 1996. Macroemulsion stability: the oriented wedge theory revisited. *Langmuir,* 12**,** 276-292.

KABALNOV, A. S. & SHCHUKIN, E. D. 1992. Ostwald ripening theory: applications to fluorocarbon emulsion stability. *Advances in Colloid and Interface Science,* 38**,** 69-97.

KANKAVI, O. 2006. HUMAN SKIN IS AS AN EXTRA-PULMONARY SITE OF SECRETION OF SURFACTANT PROTEINS. *ERCIYES TIP DERGISI,* 28**,** 082.

KAROU, D., DICKO, M. H., SIMPORE, J. & TRAORE, A. S. 2005. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. *African Journal of Biotechnology,* 4**,** 823-828.

KENNEDY, C., BASTIAENS, M. T., BAJDIK, C. D., WILLEMZE, R., WESTENDORP, R. G. & BAVINCK, J. N. B. 2003. Effect of smoking and sun on the aging skin. *Journal of investigative dermatology,* 120**,** 548-554.

KHAN, B. A., AKHTAR, N., WASEEM, K., MAHMOOD, T., RASUL, A. & IQBAL, M. 2012. Visio Scan VC98, Corneometer MPA 5 and Tewameter MPA 5. *African Journal of Pharmacy and Pharmacology,* 6**,** 225-227.

KHAZAKA, G. 2000. Information and Operating Instruction for the Visioscan VC 98 and the Software SELS 2000 (Surface Evaluation of the Living Skin). *CK Electronic, Cologne*.

KIM, D.-S., KIM, S.-Y., CHUNG, J.-H., KIM, K.-H., EUN, H.-C. & PARK, K.-C. 2002. Delayed ERK activation by ceramide reduces melanin synthesis in human melanocytes. *Cellular signalling,* 14**,** 779-785.

KIM, E., KIM, S., CHUNG, J., CHI, H., KIM, J. & CHUNG, I. 2006. Analysis of phenolic compounds and isoflavones in soybean seeds (Glycine max (L.) Merill) and sprouts grown under different conditions. *European Food Research and Technology,* 222**,** 201-208.

KLIGMAN, A., ZHENG, P. & LAVKER, R. 1985. The anatomy and pathogenesis of wrinkles. *British Journal of Dermatology,* 113, 37-42.

KOMUTARIN, T., AZADI, S., BUTTERWORTH, L., KEIL, D., CHITSOMBOON, B., SUTTAJIT, M. & MEADE, B. 2004. Extract of the seed coat of< i> Tamarindus indica</i> inhibits nitric oxide production by murine macrophages in vitro and in vivo. *Food and chemical toxicology,* 42, 649-658.

KREYDEN, O. P. & SCHEIDEGGER, E. P. 2004. Anatomy of the sweat glands, pharmacology of botulinum toxin, and distinctive syndromes associated with hyperhidrosis. *Clinics in dermatology,* 22, 40-44.

KRIS-ETHERTON, P. M., HECKER, K. D., BONANOME, A., COVAL, S. M., BINKOSKI, A. E., HILPERT, K. F., GRIEL, A. E. & ETHERTON, T. D. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American journal of medicine,* 113, 71-88.

KRSTONOŠIĆ, V., DOKIĆ, L., DOKIĆ, P. & DAPČEVIĆ, T. 2009. Effects of xanthan gum on physicochemical properties and stability of corn oil-in-water emulsions stabilized by polyoxyethylene (20) sorbitan monooleate. *Food Hydrocolloids,* 23, 2212-2218.

KRUTMANN, J. & GILCHREST, B. A. 2006. Photoaging of skin. *Skin aging.* Springer.

LAGÜES, M., OBER, R. & TAUPIN, C. 1978. Study of structure and electrical conductivity in microemulsions: evidence for percolation mechanism and phase inversion. *Journal de Physique Lettres,* 39, 487-491.

LAU, C. K. & DICKINSON, E. 2005. Instability and structural change in an aerated system containing egg albumen and invert sugar. *Food Hydrocolloids,* 19, 111-121.

LAZARUS, M. C. & BAUMANN, L. S. 2001. The use of cosmeceutical moisturizers. *Dermatologic Therapy,* 14, 200-207.

LEE, C. H., YANG, L., XU, J. Z., YEUNG, S. Y. V., HUANG, Y. & CHEN, Z.-Y. 2005. Relative antioxidant activity of soybean isoflavones and their glycosides. *Food Chemistry,* 90, 735-741.

LEE, K. & MACK, G. W. 2006. Role of nitric oxide in methacholine-induced sweating and vasodilation in human skin. *Journal of Applied Physiology,* 100, 1355-1360.

LEON L, H. A. 2009. *The Theory And Practice Of Industrial Pharmacy.*, CBS,New Delhi.

LEON L, H. A. 2009b. *The Theory and Practice of Industrial Pharmacy*, CBS, New Delhi.

LEON S, A. H., PAUL FS, LARRY NS 2004. *Comprehensive Pharmacy Review*, Lipponcott Williams And Wilkins, Philadelphia.

LI, W.-G., ZHANG, X.-Y., WU, Y.-J. & TIAN, X. 2001. Anti-inflammatory effect and mechanism of proanthocyanidins from grape seeds. *Acta Pharmacologica Sinica,* 22**,** 1117-1120.

LIEBERMAN, H. A., RIEGER, M. M. & BANKER, G. S. 1998. *Pharmaceutical Dosage Forms--Disperse Systems*, Marcel Dekker.

LING, B. & BAI-PING, F. 2008. Advances in research of grape seed proanthocyanidin extracts [J]. *Chinese Journal of New Drugs,* 17**,** 006.

LIU, W., SUN, D., LI, C., LIU, Q. & XU, J. 2006. Formation and stability of paraffin oil-in-water nano-emulsions prepared by the emulsion inversion point method. *Journal of Colloid and Interface Science,* 303**,** 557-563.

LOURITH, N., KANLAYAVATTANAKUL, M. & CHANPIROM, S. 2009. Free radical scavenging efficacy of Tamarind seed coat and its cosmetics application. *J Health Res,* 23**,** 159-162.

LUZIA, D. M. M. & JORGE, N. 2011. Antioxidant activity, fatty acid profile and tocopherols of Tamarindus indica L. seeds. *Food Science and Technology (Campinas),* 31**,** 497-501.

MAHDAVIAN, A. & SHARIFI-SANJANI, N. 2001. Role of emulsifiers in preparation of core-shell particles via step-wise emulsion polymerization. *Iranian Polymer Journal,* 10**,** 243-250.

MALENČIĆ, D., MAKSIMOVIĆ, Z., POPOVIĆ, M. & MILADINOVIĆ, J. 2008. Polyphenol contents and antioxidant activity of soybean seed extracts. *Bioresource technology,* 99**,** 6688-6691.

MARTINI, F. H. 2004. *The integumentary system. In Fundamentals of Anatomy and Pysiology* Pearson Education.

MASAKI, H. 2010. Role of antioxidants in the skin: anti-aging effects. *Journal of dermatological science,* 58**,** 85-90.

MCAULIFFE, C. D. 1973. Oil-in-water emulsions and their flow properties in porous media. *Journal of petroleum technology,* 25**,** 727-733.

MCLEAN, J. D. & KILPATRICK, P. K. 1997. Effects of asphaltene aggregation in model heptane–toluene mixtures on stability of water-in-oil emulsions. *Journal of Colloid and Interface Science,* 196**,** 23-34.

MENON, V. & WASAN, D. 1988. A review of the factors affecting the stability of solids-stabilized emulsions. *Separation Science and technology,* 23**,** 2131-2142.

MERAD, M., MANZ, M. G., KARSUNKY, H., WAGERS, A., PETERS, W., CHARO, I., WEISSMAN, I. L., CYSTER, J. G. & ENGLEMAN, E. G. 2002. Langerhans cells renew in the skin throughout life under steady-state conditions. *Nature immunology,* 3**,** 1135-1141.

MICHAEL HR, G. I., WOJCIECH P 2003. *Histology A Text Book and Atlas*, Lipponcott Williams and Wilkins, Philadelphia.

MITSUI, T. 1998. *New Cosmetic Science,* Elsevier Sciences., The Netherlands.

MITTLER, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in plant science,* 7**,** 405-410.

MOLL, I., BLADT, U. & JUNG, E. 1990. Presence of Merkel cells in sun-exposed and not sun-exposed skin: a quantitative study. *Archives of dermatological research,* 282**,** 213-216.

MOOI, W. & KRAUSZ, T. 1992. Melanin and melanocytes. *Biopsy Pathology of Melanocytic Disorders.* Springer.

MORIN, B., NARBONNE, J.-F., RIBERA, D., BADOUARD, C. & RAVANAT, J.-L. 2008. Effect of dietary fat-soluble vitamins A and E and proanthocyanidin-rich extract from grape seeds on oxidative DNA damage in rats. *Food and chemical toxicology,* 46**,** 787-796.

MORITA, A. 2007. Tobacco smoke causes premature skin aging. *Journal of dermatological science,* 48**,** 169-175.

MUHAMMAD, K. W., AKHTAR, N., HAJI, M., MUSTAFA, R. & MURTAZA, G. 2014. Stability Study of a Cosmetic Emulsion Loaded with Tamarindus indica Seeds Extract. *Lat. Am. J. Pharm,* 33**,** 731-8.

NAMBAM, J. S. & PHILIP, J. 2012. Competitive adsorption of polymer and surfactant at a liquid droplet interface and its effect on flocculation of emulsion. *Journal of colloid and interface science,* 366**,** 88-95.

NAVEED, A. 2001. *Formulation and evaluation of a cosmetic multiple emulsion system containing macademia nut oil and two antiaging agents.* Dissertation for the Degree of Doctor of Philosophy The Department of Pharmaceutical Technology,Anadolu University.

NAWAZ, H., SHI, J., MITTAL, G. S. & KAKUDA, Y. 2006. Extraction of polyphenols from grape seeds and concentration by ultrafiltration. *Separation and Purification Technology,* 48**,** 176-181.

NIELLOUD, F. 2000. *Pharmaceutical Emulsions and Suspensions: Revised and Expanded*, CRC Press.

NOHYNEK, G. J., ANTIGNAC, E., RE, T. & TOUTAIN, H. 2010. Safety assessment of personal care products/cosmetics and their ingredients. *Toxicology and applied pharmacology,* 243**,** 239-259.

NORMAN, A. W. 1998. Sunlight, season, skin pigmentation, vitamin D, and 25-hydroxyvitamin D: integral components of the vitamin D endocrine system. *The American journal of clinical nutrition,* 67**,** 1108-1110.

NOUR, A. H. & YUNUS, R. M. 2006. Stability investigation of water-in-crude oil emulsion. *Applied Sci,* 6**,** 2895-2900.

O'MAY, G. A., ALLISON, D. & GILBERT, P. 2004. A rapid method for the evaluation of both extrinsic and intrinsic contamination and resulting spoilage of water-in-oil emulsions. *Journal of applied microbiology,* 96**,** 1124-1132.

O'GOSHI, K. 2006. Optical measurement of sebum excretion using opalescent film imprint. The Sebumeter. *Handbook of Non-Invasive Methods and the Skin***,** 841-846.

OKUN, M. R., EDELSTEIN, L. M., HAMADA, G., DONNELLAN, B. & OR, N. 1970. The role of peroxidase vs. the role of tyrosinase in enzymatic conversion of tyrosine to melanin in melanocytes, mast cells and eosinophils. *Journal of Investigative Dermatology,* 55**,** 1-12.

ONUKI, A. 1993. Viscosity of emulsion with surfactant. *EPL (Europhysics Letters),* 24**,** 151.

OPAWALE, F. O. & BURGESS, D. J. 1998. Influence of interfacial properties of lipophilic surfactants on water-in-oil emulsion stability. *Journal of colloid and interface science,* 197**,** 142-150.

PAINE, C., SHARLOW, E., LIEBEL, F., EISINGER, M., SHAPIRO, S. & SEIBERG, M. 2001. An alternative approach to depigmentation by soybean extracts via inhibition of the PAR-2 pathway. *Journal of investigative dermatology,* 116**,** 587-595.

PARVEZ, S., KANG, M., CHUNG, H. S. & BAE, H. 2007. Naturally occurring tyrosinase inhibitors: mechanism and applications in skin health, cosmetics and agriculture industries. *Phytotherapy research,* 21**,** 805-816.

PEARCE, C. 2005. The electrical conductivity and permittivity of mixtures, with special reference to emulsions of water in fuel oil. *British Journal of Applied Physics,* 6**,** 113.

PENA FERREIRA, R., COSTA, P. & BAHIA, F. 2003. Visioscan VC 98 application: a comparison study between coarse and smooth surface. *Skin Res Technol,* 9**,** 204-205.

PODDA, M. & GRUNDMANN-KOLLMANN, M. 2001. Low molecular weight antioxidants and their role in skin ageing. *Clinical and experimental dermatology,* 26**,** 578-582.

PORTER, W. L., BLACK, E. D. & DROLET, A. M. 1989. Use of polyamide oxidative fluorescence test on lipid emulsions: contrast in relative effectiveness of antioxidants in bulk versus dispersed systems. *Journal of Agricultural and Food Chemistry,* 37**,** 615-624.

PÖSSEL, P., AHRENS, S. & HAUTZINGER, M. 2005. Influence of cosmetics on emotional, autonomous, endocrinological, and immune reactions. *International journal of cosmetic science,* 27**,** 343-349.

PRIMAVERA, G. & BERARDESCA, E. 2005. Clinical and instrumental evaluation of a food supplement in improving skin hydration. *International journal of cosmetic science,* 27**,** 199-204.

RAYMOND CR, P. J., PAUL JW 2003. *Dimethicone, Mineral Oil, Wax White; Wax Yellow., Handbook of Pharmaceutical Excipients*, The PhP. London, Chicago, and the APhA. Washington.

REMINGTON, J. P., TROY, D. B. & BERINGER, P. 2006. *Remington: The science and practice of pharmacy,* Lippincott Williams & Wilkins.

REUTER, J., MERFORT, I. & SCHEMPP, C. M. 2010. Botanicals in dermatology. *American journal of clinical dermatology,* 11**,** 247-267.

RG, A. 2000. *The Science and Practice of Pharmacy I*, The Parthenon: New York.

RICE-EVANS, C. A., MILLER, N. J., BOLWELL, P. G., BRAMLEY, P. M. & PRIDHAM, J. B. 1995. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free radical research,* 22**,** 375-383.

RITTIÉ, L. & FISHER, G. J. 2002. UV-light-induced signal cascades and skin aging. *Ageing research reviews,* 1**,** 705-720.

ROBINS, M. M. 2000. Emulsions—creaming phenomena. *Current opinion in colloid & interface science,* 5**,** 265-272.

ROMERO, A., CORDOBÉS, F. & GUERRERO, A. 2009. Influence of pH on linear viscoelasticity and droplet size distribution of highly concentrated O/W crayfish flour-based emulsions. *Food hydrocolloids,* 23**,** 244-252.

ROUSSEAU, D. 2000. Fat crystals and emulsion stability—a review. *Food Research International,* 33**,** 3-14.

SARAVACOS, G. & KOSTAROPOULOS, A. 1995. Transport properties in processing of fruits and vegetables. *Food Technology,* 49**,** 99-105.

SATO, M., MAULIK, G., RAY, P. S., BAGCHI, D. & DAS, D. K. 1999. Cardioprotective effects of grape seed proanthocyanidin against ischemic reperfusion injury. *Journal of molecular and cellular cardiology,* 31**,** 1289-1297.

SATO, T., SAKAMOTO, W., ODANAKA, W., YOSHIDA, K. & URUSHIBARA, O. 2002. Clinical effects of dietary hyaluronic acid on dry, rough skin. *Aesthetic Dermatology,* 12**,** 109-120.

SCHULMAN, J. H., STOECKENIUS, W. & PRINCE, L. M. 1959. Mechanism of formation and structure of micro emulsions by electron microscopy. *The Journal of Physical Chemistry,* 63**,** 1677-1680.

SHAH, J. C. 2007. Encyclopedia of Pharmaceutical Technology. 15-25.

SHARMA, S., PODDAR, R., SEN, P. & ANDREWS, J. 2008. Effect of vitamin C on collagen biosynthesis and degree of birefringence in polarization sensitive optical coherence tomography (PS-OCT). *African Journal of Biotechnology,* 7.

SHI, J., YU, J., POHORLY, J. E. & KAKUDA, Y. 2003. Polyphenolics in grape seeds-biochemistry and functionality. *Journal of medicinal food,* 6**,** 291-299.

SIDDHURAJU, P. 2007. Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated< i> Tamarindus indica</i> seed coat. *LWT-Food Science and Technology,* 40**,** 982-990.

SILVER, F. H., FREEMAN, J. W. & DEVORE, D. 2001. Viscoelastic properties of human skin and processed dermis. *Skin Research and Technology,* 7**,** 18-23.

SIMONETTI, P., CIAPPELLANO, S., GARDANA, C., BRAMATI, L. & PIETTA, P. 2002. Procyanidins from Vitis vinifera seeds: in vivo effects on oxidative stress. *Journal of agricultural and food chemistry,* 50**,** 6217-6221.

SIMPSON, N. B. & CUNLIFFE, W. J. 2004. Disorders of the sebaceous glands. *Rook's Textbook of Dermatology, Seventh Edition***,** 2121-2196.

SLOMINSKI, A., TOBIN, D. J., SHIBAHARA, S. & WORTSMAN, J. 2004. Melanin pigmentation in mammalian skin and its hormonal regulation. *Physiological reviews,* 84**,** 1155-1228.

SOURLA, A., RICHARD, V., LABRIE, F. & LABRIE, C. 2000. Exclusive androgenic effect of dehydroepiandrosterone in sebaceous glands of rat skin. *Journal of endocrinology,* 166**,** 455-462.

STALLINGS, A. F. & LUPO, M. P. 2009. Practical uses of botanicals in skin care. *The Journal of clinical and aesthetic dermatology,* 2**,** 36.

STEWART, M. Sebaceous gland lipids. Seminars in dermatology, 1992. 100-105.

SÜDEL, K. M., VENZKE, K., MIELKE, H., BREITENBACH, U., MUNDT, C., JASPERS, S., KOOP, U., SAUERMANN, K., KNUBMANN-HARTIG, E. & MOLL, I. 2005. Novel Aspects of Intrinsic and Extrinsic Aging of Human Skin: Beneficial Effects of Soy Extract¶. *Photochemistry and photobiology,* 81**,** 581-587.

TAL-FIGIEL, B. 2007. The formation of stable w/o, o/w, w/o/w cosmetic emulsions in an ultrasonic field. *Chemical Engineering Research and Design,* 85**,** 730-734.

TAMBURIC, S., CRAIG, D., VULETA, G. & MILIC, J. 1996. An investigation into the use of thermorheology and texture analysis in the evaluation of w/o creams stabilized with a silicone emulsifier. *Pharmaceutical development and technology,* 1**,** 299-306.

TEMPFER, C. B., BENTZ, E.-K., LEODOLTER, S., TSCHERNE, G., REUSS, F., CROSS, H. S. & HUBER, J. C. 2007. Phytoestrogens in clinical practice: a review of the literature. *Fertility and sterility,* 87**,** 1243-1249.

THONGMUANG, P. & SUDJAROEN, Y. The Tyrosinase and Cyclooxygenase Inhibitory Activities and Cytotoxicity Screening of Tamarindus indica Seeds. *seed,* 50**,** 12.5.

THORNFELDT, C. 2005. Cosmeceuticals containing herbs: fact, fiction, and future. *Dermatologic Surgery,* 31**,** 873-881.

TOBIN, D. J. 2006. Biochemistry of human skin—our brain on the outside. *Chemical Society Reviews,* 35**,** 52-67.

TSUDA, T., WATANABE, M., OHSHIMA, K., YAMAMOTO, A., KAWAKISHI, S. & OSAWA, T. 1994. Antioxidative components isolated from the seed of tamarind (Tamarindus indica L.). *Journal of Agricultural and Food Chemistry,* 42**,** 2671-2674.

UITTO, J. 2008. The role of elastin and collagen in cutaneous aging: intrinsic aging versus photoexposure. *Journal of drugs in dermatology: JDD,* 7**,** s12-6.

VALKO, M., LEIBFRITZ, D., MONCOL, J., CRONIN, M. T., MAZUR, M. & TELSER, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology,* 39**,** 44-84.

VIYOCH, J., KLINTHONG, N. & SIRIPAISAL, W. 2013. Development of oil-in-water emulsion containing Tamarind fruit pulp extract I. Physical characteristics and stability of emulsion. *วารสาร มหาวิทยาลัย นเรศวร (Naresuan University Journal),* 11**,** 29-44.

VOEGELI, D. 2009. The role of emollients in the care of patients with dry skin. *RCNP Journal Supplements,* 3**,** 21-27.

VYAS, S. P. & KHAR, R. K. 2004. *Targeted & Controlled Drug Delivery: Novel Carrier Systems*, CBS publishers & distributors.

WANG, D., ZHANG, H., ARENS, E. & HUIZENGA, C. 2007. Observations of upper-extremity skin temperature and corresponding overall-body thermal sensations and comfort. *Building and Environment,* 42**,** 3933-3943.

WAQAS, M., AKHTAR, N., RASUL, A., RASHID, S., MUSTAFA, R., KHAN, B. & MURTAZA, G. 2014. In vivo Evaluation of a Cosmetic Emulsion Containing Soybean Extract for Anti-Aging. *Tropical Journal of Pharmaceutical Research,* 13**,** 1401-1406.

WAQAS, M. K., AKHTAR, N., AHMAD, M., MURTAZA, G., KHAN, H., IQBAL, M., RASUL, A. & BHATTI, N. S. 2010. Formulation and characterization of a cream containing extract of fenugreek seeds. *Acta Pol Pharm,* 67**,** 173-178.

WAQAS, M. K., SHAH, A., AKHTAR, N. & MURTAZA, G. 2013. Screening of various botanical extracts for antioxidant activity using DPPH free radical method. *African Journal of Traditional, Complementary and Alternative Medicines,* 10**,** 452-455.

WEI, H., SALADI, R., LU, Y., WANG, Y., PALEP, S. R., MOORE, J., PHELPS, R., SHYONG, E. & LEBWOHL, M. G. 2003. Isoflavone genistein: photoprotection and clinical implications in dermatology. *The Journal of nutrition,* 133**,** 3811S-3819S.

WEISSENBORN, P. & MOTIEJAUSKAITE, A. 2000. Emulsification, drying and film formation of alkyd emulsions. *Progress in organic coatings,* 40**,** 253-266.

YAAR, M. & GILCHREST, B. A. 2007. Photoageing: mechanism, prevention and therapy. *British Journal of Dermatology,* 157**,** 874-887.

YAMAKOSHI, J., OTSUKA, F., SANO, A., TOKUTAKE, S., SAITO, M., KIKUCHI, M. & KUBOTA, Y. 2003. Lightening Effect on Ultraviolet-Induced Pigmentation of Guinea Pig Skin by Oral Administration of a Proanthocyanidin-Rich Extract from Grape Seeds. *Pigment Cell Research,* 16**,** 629-638.

YILMAZ, E. & BORCHERT, H.-H. 2006. Effect of lipid-containing, positively charged nanoemulsions on skin hydration, elasticity and erythema—an in vivo study. *International journal of pharmaceutics,* 307**,** 232-238.

YOKOZAWA, T., LEE, Y. A., CHO, E. J., MATSUMOTO, K., PARK, C. H. & SHIBAHARA, N. 2011. Anti-aging effects of oligomeric proanthocyanidins isolated from persimmon fruits. *Drug discoveries & therapeutics,* 5**,** 109-118.

YOSIPOVITCH, G., TANG, M., DAWN, A. G., CHEN, M., GOH, C. L., CHAN, Y. H. & SENG, L. F. 2007. Study of psychological stress, sebum production and acne vulgaris in adolescents. *Acta dermato-venereologica,* 87**,** 135-139.

ZHU, W. & GAO, J. The use of botanical extracts as topical skin-lightening agents for the improvement of skin pigmentation disorders. Journal of Investigative Dermatology Symposium Proceedings, 2008. Nature Publishing Group, 20-24.

ZI, S.-X., MA, H.-J., LI, Y., LIU, W., YANG, Q.-Q., ZHAO, G. & LIAN, S. 2009. Oligomeric proanthocyanidins from grape seeds effectively inhibit ultraviolet-induced melanogenesis of human melanocytes in vitro. *International journal of molecular medicine,* 23**,** 197-204.

ZOUBOULIS, C. C. 2004. Acne and sebaceous gland function. *Clinics in dermatology,* 22**,** 360-366.